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SOME ASPECTS OF BASIDIOCARP MORPHOGENESIS IN  
*Gloeophyllum (Lenzites) saepiarium* (Wulf.) Karsten,  
A XEROPHYTIC POLYPORE

by



JACK STERLING STATES

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "SOME ASPECTS OF BASIDIOCARP MORPHOGENESIS IN *Gloeophyllum* (*Lenzites*) *saepiarium* (Wulf.) Karsten, A XEROPHYTIC POLYPORE", submitted by Jack Sterling States in partial fulfilment of the requirements for the degree of Doctor of Philosophy.



## ABSTRACT

The development of *Gloeophyllum saepiarium* in the natural habitat and in culture has been described. In nature, the differentiation of hyphae to form basidiocarps occurs in direct response to the environmental conditions present during development. The initial control of basidiocarp development is of a genetic nature and was found to be related to the establishment of a dikaryotic condition in the vegetative hyphae. The environment plays a formative role in the execution of the genetic potential. As a consequence, *G. saepiarium* exhibits in its developmental morphology perfect adaptations for survival and reproduction under the changing, and often severe conditions that characterize its habitat. The environmental factors of primary importance in normal basidiocarp development have been found to be: light to initiate a fruiting response, available substrate moisture and a fluctuating atmospheric humidity to induce periodic desiccation of apical hyphae. A marked alteration of environmental conditions, to which specific adaptations have been made, results in the failure of the mycelium to respond in an appropriate fashion according to its developmental pattern. Such changes are inherent in standard cultural conditions and are found to some extent in the natural habitat. They are responsible for considerable abnormality of microstructure and physiognomy in both vegetative and reproductive stages of the life cycle. Normal basidiocarps form in culture only when the general conditions of the natural environment are provided. A general consistency in the polymorphic response of the mycelium to environmental variables exists because of the limits imposed by the genetic constitution. Within this framework the individuality of





*G. saepiarium* has been defined. The relevance of the information obtained in the study to the taxonomy and morphogenesis of other fungi is discussed.



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## INTRODUCTION

Basidiocarp characters used in the classification of the Polyporaceae have been the subject of considerable debate among mycologists. The gross morphology of the basidiocarp used in comparative studies has, traditionally, provided the basis for the taxonomic treatment of this heterogeneous group of basidiomycetes. Many workers consider this approach unsatisfactory since it leads to an artificial assemblage of unrelated species. The pronounced polymorphology and extreme complexity of the basidiocarp in many species account for the difficulty in the recognition of distinct characters which would set species apart yet satisfactorily bind them in related groups at the generic level.

More recently, attempts have been made to discover and select characters which would provide a more natural system of classification. An excellent summary and critical evaluation of these attempts has been presented by Bondartseva (1961). Hyphal features and arrangements have received particular emphasis but are not entirely satisfactory for the purposes of classification.

Corner (1953) and Cunningham (1954) grouped taxa with structural affinities according to the presence or absence of different hyphal types, the so-called "mitic" systems. Unfortunately, the recognition of these systems is difficult particularly for the inexperienced worker. There is also an apparent latitude in the terminology used to define the systems (Pinto-Lopes, 1952). Anatomical characters which have been considered most important in the delimitation of taxa in other systems include the presence or absence of clamp connections, color and thickness of the hyphal walls, modifications in terminal cells and septation. As Smith (1968, p. 743 ) has noted, these features are often variable



and placing undue emphasis on one or more of them for use in a taxonomy can lead to "making the same mistakes as our predecessors only using different sets of characters in the process".

Perhaps one of the most significant contributions to the establishment of natural relationships within the Polyporaceae has been the study of biochemical and morphological cultural characters by Nobles (1958, 1965). Enzyme systems known to be characteristic of brown rot and white rot wood-decay fungi provided a new and primary separation of two apparently distinct groups of polypores. She made no attempt to segregate genera on the basis of cultural characters, and rightly so, since relationships between cultural characters and those expressed in the natural environment have not been established. Comparative studies are needed to determine the taxonomic significance of cultural characters.

The reliability of basidiocarp characters for use in a taxonomy is suspect because of their variability, not only within genera but in the same species. These characters should provide a natural system of classification since they are products of an internal process genetically regulated. It is my hypothesis that much of the morphological variability in polypores is environmentally induced. Environmental influence is especially important in this group of fungi because of their mode of growth and development.

Unlike the rapid, determinate growth exhibited by agaric fructifications which mature by inflation and rearrangement of previously formed cells in a primordium, the basidiocarps of polypores develop slowly. The tough, persistent flesh of the pileus is laid down at the growing margin by apical extension and branching of hyphae to form new cells. Continued growth is often intermittent and perennial producing a





characteristic zonation of the pileus surface. The basidiocarp mycelium is exposed to a variety of environmental conditions for long periods of time. During development individual hyphae and groups of hyphae are presumably in stages of transition which may be interrupted or modified by the environment before maturity is reached. Morphological sensitivity to environmental conditions is evident in artificial culture as basidiocarps that are produced are abnormal. Long and Harsch (1918, p. 76) remarked that "in all the thousands of cultures of polypores with the hundreds of sporophores produced, not a single one had a typical pileus". Attempts in the laboratory to induce basidiocarps similar to those found in nature have been unsuccessful for most species tested.

I consider standard cultural conditions to be inherently artificial since they fail to provide the necessary environmental conditions for normal basidiocarp development, even if the genetic potential to produce normal basidiocarp development is present. The determination of environmentally induced variation in basidiocarp morphology can be made by a careful study of hyphal characters which comprise the developmental stages. A comparative study of characters expressed in the natural habitat with those in artificial culture would be of great value in establishing the taxonomic significance of cultural characters.

There have been few studies dealing with the developmental (ontogenetic) aspects of microstructural characters in polypores. A descriptive study of sequential changes in form as influenced by both genetic and environmental conditions can contribute much needed information in regard to taxonomic characters and integrate aspects of morphogenesis into a more complete understanding of basidiocarp development. The objective of this study was to provide a detailed description of



basidiocarp development of a wood-decay polypore, in nature and in culture, and to determine the influence of the external environment on the developmental characteristics.

Few species in the Polyporaceae are better suited for a study of this type than *Gloeophyllum (Lenzites) saepiarium* (Wulf.) Karsten. It is a bracket fungus which, as a xerophyte, possesses a marked degree of hyphal differentiation, is found in many different ecological habitats, and has received considerable attention in the literature because of its importance in wood-decay. The abundant basidiocarps produced by this polypore in culture are consistently abnormal. Irregularly formed fructifications have also been noted in the natural environment. As will be pointed out later, both physiological and morphological characters of *G. saepiarium* are variable under cultural conditions, as indicated by inconsistencies in data from previous papers. Anatomical descriptions of the natural basidiocarps have been presented in several studies, but I have found that the complex structure has never been correctly described. In addition, it is not known whether cultural basidiocarps are similar to natural basidiocarps in terms of their structural elements and the interrelationships of these elements. The perennial habit and polymorphic, gill-bearing basidiocarp of this fungus affords a unique opportunity to investigate the influences of environment on its developmental stages and to correlate microstructural characters produced in both natural and cultural conditions.

The exploratory nature of the several experiments conducted in this study makes it difficult to follow the usual method of data presentation. This is especially true where the results of one experiment influence the course of subsequent investigation. Therefore, results and their





discussion are more conveniently included together in many cases.

The thesis is organized in the following manner. Developmental studies of other polypores and the methods used to culture them are reviewed. Then a review of the existing literature on the biology of *G. saepiarium* is presented. The review is followed by a discussion of the natural habitat of the fungus and description of the developmental morphology as influenced by environmental conditions. The cultural aspects of growth and development are then considered. Information from the cultural study is presented in two parts. First, the relationship of the sexual incompatibility factors of inoculum source to fruiting ability is considered. Second, variations in growth and development under a number of cultural conditions are recorded and a description of the developmental morphology of basidiocarps produced under cultural conditions is presented. Finally, information obtained in the natural and cultural environments is synthesized and discussed. A conscientious effort was made to relate the results to aspects of morphogenesis in other polypores in the discussion so that, hopefully, a significant contribution would be made to our knowledge of growth mechanics in these fungi as well as to the evaluation of characters used in their taxonomy.



## LITERATURE REVIEW

Developmental Studies of the Polyporaceae

The impetus to study life cycles of polypores has come from their economic importance as wood-decay fungi. The literature abounds with studies on the biology of important wood-decay species but these treatments are primarily concerned with the vegetative stages in the wood and the reproductive capacities of the sexual and asexual stages. Of importance to this study are those investigations which deal specifically with the anatomic characteristics of the basidiocarps and the environmental factors which have a formative influence on their development. Studies of this nature were scattered in the literature, but when assembled were of great value to the present study.

In 1909, Falck published a detailed account of the life cycles of *Lenzites abietina*, *L. saepiaria* and *L. thermophila*. Because of the pronounced polymorphology of the basidiocarps, he considered in great detail their structure as formed in cultural and natural environments. He concluded that the differently formed fruit bodies were ontogenetic stages interrupted by conditions of the environment. The most primitive stages of development were represented by irpicoid or hypochnoid fruit bodies and the most advanced stages by pileate basidiocarps with uniform lamellae. The expression of the ontogenetic stages was influenced by factors of light, temperature and moisture as well as by position in which development took place. He devised a complex classification system to include the various fruit bodies that he encountered. A summary of his classification is given in Appendix I of this work.

By means of comparison, Falck concluded that the hyphae in artificial





culture were different from those found in natural wood. Three systems of mycelium were distinguished according to their structural characters, differences in culture and in nature, and ability to undergo further differentiation. These were: primary mycelium, the germination mycelium; secondary mycelium, the vegetative mycelium in culture (substrate mycelium) and in wood (surface hyphae); tertiary mycelium, the differentiated mycelium arising from the secondary mycelium to form specialized tissues of the conduction mycelium, the fiber mycelium of the fruit body, and the cuticular mycelium of the wood. His views on hyphal differentiation have been called into question by Walek-Czernecka (1933), but when considered within the framework of present-day terminology and knowledge of fungal morphogenesis, his observations contribute much useful information on aspects of basidiocarp development. No useful purpose would be served to dwell on the details of his investigation but appropriate references will be made to his work throughout the study.

Buller (1909) investigated the macroscopic aspects of basidiocarp structure and arrangement of the hymenium in *Polyporus squamosus*. This fungus formed abnormal fructifications in the dark, many of them having the appearance of a "stag's horn", but formed normal stipitate, pileate fruit bodies if briefly exposed to light. Once initiated, the abnormal fructifications failed to respond to unilateral illumination. He also reported that the fruit bodies orient their pilei at right angles to the force of gravity by a geotropic curvature of the stipe. The dissepiments are then formed parallel to the force of gravity by a positive geotropic response. No account was given of environmental factors provided during the study other than light and the use of infected wood which readily produced fruit bodies.



Corner (1932a) analyzed in detail the structural changes in the mycelium of *Polystictus xanthopus*, a stipitate polypore, during basidiocarp development. He found the mycelium of the basidiocarp to be composed of four systems of differentiated hyphae which he termed generative, skeletal, mediate and binding hyphae. To each type he assigned a function reflected in their epithet. The arrangement of the hyphal system in the formation of the stipe and pileus was described in detail for basidiocarps that formed from infected sticks kept continuously moist under a bell jar. The stipe was found to be positively phototropic with no indication of a response to gravity. The pileus was oriented in a horizontal plane to the earth's surface and the pore tubes were vertically disposed beneath the limb of the pileus.

In another study Corner (1932b) studied the developmental morphology of the basidiocarps of *Fomes levigatus*, a bracket fungus, and several closely related species of *Fomes*. Two hyphal systems, generative and skeletal, were found to compose the developing fruit bodies. The response of the hyphal elements was believed to be geotropic and never phototropic as was the stipe in *P. xanthopus*. The developmental sequence also varied to some extent. The developmental aspects of these fungi will be considered in the discussion of the present study. With the exception of Falck's study (1909), Corner's work is the only descriptive account of the developmental morphology of normal basidiocarps. The generic differences in the hyphal composition of the basidiocarps led Corner (1953) to formulate the now distinguished mitic system concept. In this concept the presence of generative hyphae alone is termed a monomitic system, the presence of generative and either skeletal or binding hyphae is termed a dimitic system, and the presence of all three







types of hyphae is termed a trimitic system. The use of hyphal systems as an aid to identification of polypores had been generally accepted as a valuable taxonomic criterion.

Many investigations were stimulated by the desirability of obtaining basidiocarps of polypores in culture for both developmental and taxonomic studies. Long and Harsch (1918) examined the macroscopic and microscopic characteristics of more than thirty species of polypores cultured on agar slants with different nutrient sources. The study was concerned with the identification of wood-decay fungi on the basis of cultural characteristics, particularly the reproductive structures. All fructifications produced were abnormal in shape and size, but the dimensional characteristics of the hymenophores were found to be identical to those found in nature. They concluded that the fruit bodies of the Polyporaceae were strongly phototropic in culture. The hymenophores were, however, geotropic in that they always formed parallel to gravitational forces irrespective of the incidence of light. Light was found to be necessary to initiate the fruiting response of the mycelium. No study was made of the structural elements which composed the fruit bodies.

Hopp (1938) inoculated wood blocks with *Fomes applanatus*, allowed them to become well decayed by the mycelium and placed them in each of five aerated chambers with controlled relative humidities of 35, 45, 53, 75 and 100 %. Typical sporophores were produced in the chambers maintained at 75%. Abnormal fructifications developed under a relative humidity of 100%, and no fructifications were formed at the lower humidities nor did growth appear on the surface of the blocks.

One of the problems peculiar to the study of polypores in culture was the inconvenience caused by the great length of time required by some



species to initiate fruit body formation. Badcock(1941) prepared a special decoction to accelerate the vegetative growth of species which had not previously fruited in culture. The preparation when added to sawdust and placed in various types of culture vessels was successful in shortening the period of fructification as well as inducing fruit body formation in species which had not fruited on other media. Although several basidiocarps were produced which were sufficiently typical of the species to be recognized, the normal development of basidiocarps did not occur (Badcock, 1943).

Lohwag (1955) using accelerator medium in sawdust contained in canning jars was able to obtain nearly normal fructifications of *Lenzites betulina*. He provided a narrow opening for the egress of mycelium from the culture jar to the outside with a lid loosely fitted on the threads of the neck of the jar. The culture vessel was considered similar to a natural wood stump and the space between the threads similar to dry cracks in the wood through which the hyphae could be conducted and proceed to normal fruit body formation. Unfortunately, he gave no details on the cultural environment, but did mention that in order to obtain fruit bodies in culture it was best to provide conditions closest to those in the natural environment.

A series of experiments using an apparatus to provide continuous aeration while exercising control on other environmental variables were conducted by Plunkett (1956, 1958, 1961). Using the stipitate polypore *Polyporus brumalis*, he not only obtained normal fructifications using his ingenious culture apparatus, but demonstrated the influence of several environmental factors on basidiocarp development. The expansion of the stipe by secondary inflation of cells was reported and indicated,





along with other growth characteristics, that this fungus has a different developmental pattern from polypores previously described. Its development is similar to that of agaric fructifications. Cultures with dry air provided the maximum amount of evaporation and allowed the most normal and abundant fruit bodies to be produced. The results of Plunkett's experiments are presented in a succinct review of basidiocarp morphogenesis by Taber (1966) and will be referred to later in the study.

Tamblyn and DaCosta (1958) devised a method for obtaining basidiocarps of polypores in culture by combining aspects of techniques used by Lohwag and Badcock. A complex mixture of sawdust, enriched with a number of nutrients in addition to those of the Badcock accelerator medium, was added to jars fitted with a wood block. The mycelium of the test fungus was placed on the sawdust and allowed to grow through the wood block. Fruit bodies were produced on the surface of the wood, several similar to those found in nature. No structural detail of the basidiocarps formed was mentioned, nor were specific environmental factors discussed.

The methods of culture used in the investigations reviewed above were employed in the present study. The developmental aspects of basidiocarps produced in the natural environment and in culture by *G. saepiarium* during the study, will be discussed with reference to the papers reviewed.

### *Gloeophyllum saepiarium* (Wulf.) Karsten

#### 1. Classification

According to Spaulding (1911), *Gloeophyllum saepiarium* was first described in 1786 by Von Wulfen who named it *Agaricus saepiarius*. It



was later transferred to the genus *Merulius* by Persoon in 1800. Fries first considered the species to belong to the genus *Daedalea* but later placed it in *Lenzites* naming it *Lenzites saepiaria* in 1889. According to Donk (1960) the genus *Lenzites* was erected by Fries in honor of F. A. Lenz and was typified by *Daedalea betulina* (L.) Fr. The specific membership includes forms which possess a tough, coriaceous basidiocarp with predominantly lamellate hymenophores. Karsten (1886) at first accepted this classification, but later erected the genus *Gloeophyllum* selecting *Lenzites saepiaria* as the type species. This genus was established to separate species of *Lenzites* with brown pigmented basidiocarps from those possessing little or no pigment. According to Spaulding (1911) Murrill in 1905 recognized Karsten's separation, but at first considered the type species to be *Agaricus hirsutus*. Later in 1908 he changed it to *Gloeophyllum hirsutum*. Fries in 1821 considered *Agaricus hirsutus* and *Lenzites saepiaria* to be one and the same fungus. I recognize the division of the genus *Lenzites* by Karsten, and I consider the valid binomial to be *Gloeophyllum saepiarium*. Other member species of the genus include *G. abietinum*, *G. striatum*, *G. Berkeleyi*, *G. subferruginum* and *G. trabeum*.

## 2. Distribution

The records of the geographic distribution of *G. saepiarium* show it to be present throughout Europe, Asia, the Americas and Australia in areas of coniferous vegetation and places where coniferous products are used. It is considered the fungus most destructive to coniferous wood in North America (Zeller, 1916). Studies, primarily concerned with methods of prevention and control of its wood-decay activity are frequent





in the literature. It is not restricted to the decay of softwoods. Many genera of hardwoods including the tropical green heart, a member of the laurel family with the reputation for extreme durability, have been seriously decayed by *G. saepiarium*. Extensive lists of substrata on which it has been found have been published by Spaulding (1911) and Duncan and Lombard (1965).

### 3. The mycelium

The xerophytic nature of the fungus is reflected in the habitats in which it is found and in its adaptations for survival under severe environmental conditions. It is found in exposed habitats of varying elevation and is often the only observable fungus in sites of intense insolation and high evaporation-precipitation ratios (Weir, 1926). Spaulding (1911) dried fertile basidiocarps for two years and observed them to shed spores when they were moistened. Falck (1909) attributed the nature of this remarkable xerophytic behavior to the loose construction of the mycelium of the pileus surface which readily absorbs moisture by capillarity and loses it quickly during dry conditions. Buller (1909) also noted that rapid desiccation of fruit bodies allowed the mycelium to retain its vitality whereas slow or partial desiccation resulted in its death.

The vegetative mycelium has been found to have a high temperature tolerance as well as the ability to survive under extremely dry conditions. Cartwright and Findlay (1958) found that the mycelium remained viable up to ten years in wood with a 12% moisture content, a considerably longer period than other fungi investigated. Mizumoto (1964) reported decay of pine wood containing as little as 10-12% moisture.



Loman (1965) showed that the predominance of mycelium of *G. saepiarium* in the upper portions of exposed logging slash over that of other fungi in the same wood, was directly related to its high temperature tolerance and optima for growth. It survived for 60 minutes in dry wood at 66° C and for 110 minutes in wet wood with the same temperature. Falck (1909) obtained viable mycelium from wood exposed to 97° C for two hours. Reports on temperature optima for growth vary but fall within a range of 32-34° C in culture. The maximum temperature allowing growth was found to lie between 40 and 45° C. All species of the genus exhibit high temperature optima for growth (Mizumoto, 1956b). According to Cartwright and Findlay (1958) temperature optima for fungi on wood were approximately the same as when measuring their growth on agar.

Spaulding (1911) observed the mycelium from germinated spores to enter the wood where it was cut across the grain and in small breaks in the fibers. Season cracks in the wood provided a favorable humidity for germination and mechanical breaks for the entry of hyphae. Both Falck (1909) and Zeller (1916) noted that the mycelium disappeared from the surface of the wood as moisture was lost. Hyphae were found thereafter in the tracheids, growing lengthwise along these cells. These hyphae occasionally passed through bordered pits and directly through the walls into adjacent tracheids. Zeller (1916) demonstrated the presence of diastase, ligninase, cellulase, hemicellulase and pectinase in vegetative mycelium in culture, while tests for pectase and lactase were negative.

Zeller (1917) found that the mycelium was resistant to high concentrations of resin in agar media, much higher than those found in wood, and concluded that this compound in the wood provided little natural resistance to infections. Spaulding (1911) witnessed the





formation of basidiocarps on a structural timber within five months after it had been hewn from the living tree, attesting to the rapid invasion of the wood by the vegetative mycelium.

*G. saepiarium* is characterized as a "brown rot", a fungus which attacks the cellulose fraction of the wood substrate in preference to the lignin portion. Nobles (1958) reported that tests for phenol oxidizing extracellular enzymes necessary for lignin decomposition were negative for *G. saepiarium* thus verifying its brown rot character. Although this classification is generally accepted, some doubt arises due to the observation that in the final stages of decay there is evidence of dissolution of the lignin portion of the wood. Apenitis and co-workers (1951) studied the effect of the decay of *G. saepiarium* on pine wood. The cellulose was almost completely decomposed. The lignin content, in general, was always lower than that calculated from loss in weight from extraction from sound wood showing that a considerable amount was rendered soluble by fungal attack. Higuchi (1954) demonstrated that *G. saepiarium* had enzymes capable of oxidizing tyrosine and para-amino benzoic acid and Zeller (1916) reported the presence of ligninase. The methoxyl content of spruce wood decreased linearly with degree of disintegration of the wood by *G. saepiarium* according to Grohn and Deters (1959). The alteration of methoxyl content was thought to be due to enzyme activity effecting a dissolution of the lignin fraction associated chemically or physically with the degraded cellulose. The results of these studies cast doubt on the reported failure of *G. saepiarium* to break down lignin because of a "so-called" selective cellulose-decomposing enzyme system.

Both Falck (1909) and Spaulding (1911) have presented detailed



descriptions of the character of the rot and of control measures used to preserve the wood. It may be said that, in general, the heartwood is not attacked, perhaps only in advanced stages of decay, but that the sapwood is often completely rotted and reduced to a brown powder. In any case, the wood is darkened where the fungus has invaded the tissue and the mycelium may persist in its activity for many years.

#### 4. The basidiospores

The basidiospores are abundantly produced and maintain viability upon desiccation. Germination of basidiospores, desiccated for two years, was reported by Snell (1922). Exposure to direct sunlight for more than four hours was fatal. Germination was enhanced by high relative humidity, but prolonged exposure to saturated conditions decreases viability (Mizumoto, 1958). According to Walkinshaw and Scheld (1965), the percentage of germination is low in distilled water and is increased by anaerobic conditions. Further germ tube growth was inhibited at low oxygen levels. Morton and French (1966) conducted a detailed study on factors influencing spore germination on wood. The highest percentage of germination (50%) of basidiospores took place at 27° C to 35° C with no differences between the temperatures after 24 hours. Number of spores per unit area did not influence germination. Contrary to the report of Mizumoto (1958), germination increased when spores were soaked for five days but the time required for germination was longer.

The ultrastructure of the basidiospores and mycelium of two-day old cultures of *G. saepiarium* has been examined by Hyde and Walkinshaw (1966). The cytoplasm in the mycelium was granular and highly vacuolate, particularly in older portions of the hyphal elements. In





comparison, the cytoplasm in spores was more or less uniform in consistency. One or two nuclei were present in the spores. Greater numbers of mitochondria were found in germinating spores than in resting spores. Elaborate membrane systems were associated with the cell membrane and the dolipore septum in the mycelium. The membrane septa were postulated to be associated with formation of lomasomes and the hydrolytic enzymes for utilization of the wood. The vacuolization was presumed to be a degeneration process which might result in the conversion of hyphal elements to conduction tubes.

## 5. Cultural studies

Several of the physiological aspects of *G. saepiarium* have been studied in culture. Robbins (1951) using a basal synthetic medium found that it grew poorly unless supplemented with vitamins, biotin, thiamine and casein hydrolysate. Better growth was found on 2% malt extract with the addition of yeast extract, which indicated the importance of vitamins in the nutrition of this fungus. Kogel and Fries (in Wolf and Wolf, 1947, p. 26) noted that mycelial growth rates were more stimulated by the addition of thiamine to the medium than when biotin was added. Hacskeylo, et al. (1954) reported poor growth on Richard's solution where the nitrogen was supplied as ammonium nitrogen or nitrate nitrogen. Growth was accelerated by the addition of asparagine. No fructifications were reported to form on any defined medium.

The pH of all media used in the cultural studies was consistently lowered by the metabolic activity of *G. saepiarium* (Cartwright and Findlay, 1958). Mizumoto (1959) established that the optimum pH on malt extract medium was 5.0-5.5 with a range of 2.0-7.0. A pH shift from



an initial pH of 7.0 to 3.1-3.6 was noted over a 30 day period where it remained constant. Wolpert (1928) reported a pH tolerance range of 3.4-7.3 on Richard's solution and 2.8-7.5 on peptone-dextrose media. Optimum pH levels of growth on basal synthetic media were found to lie between 4.0 and 5.2 (Hacskeylo et al., 1954). A gradual decrease in growth rates was noted as the pH was artificially raised from acid to neutral.

Mizumoto (1956b) reported the optimum temperature for growth on Richard's solution to be within the range of 32-36° C. He also studied the effect of light on growth on this medium. High light intensities were harmful to fungus growth but moderate and low light intensities favored growth, especially when provided with the violet portion of the spectrum.

Growth on various carbon sources was found to be variable (Mizumoto, 1956a). When sucrose, glucose, fructose, galactose, maltose and starch were added to Richard's solution, growth rates increased in that order. *G. saepiarium* was unable to decompose filter paper cellulose in a minimal potato extract solution. A great difference in the ability to decay colloidal cellulose was noted between inoculum from the same source and from different isolates.

*G. saepiarium* was grown on malt extract, corn meal, and prune agar media in experiments conducted by Long and Harsch (1918). In every case fruit bodies that were abnormal in form were produced in 20 to 30 days. In the studies of Falck (1909), Zeller (1916) and Walek-Czernecka (1933), in which both malt extract agar and natural wood medium were used, fruiting was reported to be abnormal. Cartwright (1931) attributed the absence of a pileus in all abnormal fruit bodies to a sacrifice of





sterile tissue to fertile tissue under cultural conditions. Cauchon (1963) observed a more normal and abundant fruiting on agar slants that were placed in a downward position to allow development in response to gravity.

The descriptions of the cultural characters of the vegetative mycelium and subsequent fruit body formation by the authors cited above, essentially match those that I present later in the study. However, it is appropriate to mention those characteristics which Nobles (1965) has considered of taxonomic importance in the identification of *G. saepiarium* in culture. A character considered to be of primary importance is the negative result in tests for extracellular oxidase. A second distinguishing character is the consistently nodose-septate vegetative mycelium (clamped mycelium). Hyphal differentiation to form skeletal and binding hyphae was found to be variable, but the production of oidia, chlamydospores, and a bright yellow or orange mycelial mat allowed a separation of *G. saepiarium* from the major portion of polypores studied. Where differentiated hyphae were absent, the production of oidia, a brightly colored mat and scarcity of chlamydospores served to separate *G. saepiarium* from *G. trabeum*, which has abundant chlamydospores, and from *Trametes odorata* which has a dull yellow mat. Odor is also reported to be distinctive in all three species. Characteristics which this species has in common with other species are: ellipsoid or cylindrical basidiospores, coloration of the reverse plate, a variable growth rate, decay of both coniferous and deciduous woods and a heterothallic, bipolar type of interfertility.

Mounce and Macrae(1936) studied the sexual incompatibility factors of *G. saepiarium* using monospore isolates derived from single





basidiospores of two separate basidiocarps. A heterothallic, bipolar pattern of interfertility was obtained. Clamp connections were formed in every compatible pairing in one series of matings where this reaction was expected to occur on the basis of bipolarity. However, the second set of matings was less uniform in its reaction, a condition attributed to the age of the sporocarp from which the basidiospores had been obtained. Pairings of oidia resulted in mating reactions similar to those of the haploid mycelium.

David (1968) studied the cultural characteristics and nuclear behavior of the vegetative mycelium of monokaryotic and dikaryotic mycelia. Germ tubes emerging from binucleate basidiospores were multinucleate for some time, even after septum formation occurred. Oidia found on monokaryotic mycelium contained one or two nuclei, more rarely three or four. The dikaryotic mycelium arising from oidia was found to be multinucleate. Most of the clamped mycelia had a binucleate condition, but under anaerobic conditions of submerged mycelium in liquid agar clamps degenerated and failed to form. When the mycelium was returned to aerobic conditions clamp connections formed again. Germination mycelia from oidia produced on dikaryotic mycelia were also multinucleate and, at first, were simple septate but later formed clamps with binucleate cells.



## THE NATURAL ENVIRONMENT AND BASIDIOCARP DEVELOPMENT

Introduction

It is not surprising that a considerable degree of variability is encountered when one views fully developed basidiocarps in a natural habitat. External morphological modifications reflect the constant and selective pressures of the environment and the adjustments made by the fungus to those pressures. A question arises as to whether the morphological plasticity of *G. saepiarium* is due to the nature of its genetic constitution, or merely the influence of environmental factors on the subsequent development of genetically constant characters. In all probability, both of these factors are involved. The separation of morphological responses due to environmental stimuli (nongenetic) from those due to genetic control is difficult since it is hard to exercise experimental control on these variables in a natural habitat. However, it should be possible to characterize *G. saepiarium* if the sum of its morphological characteristics is considered with regard to the range of variation in natural and cultural environments.

In this portion of the study, collections of basidiocarps from different geographical areas and substrata were analyzed for variation in construction. Since other fungi occurring concomitantly on the same substratum could possibly influence the growth responses of *G. saepiarium*, their presence was also taken into consideration. Records were kept of general environmental conditions during progressive stages of basidiocarp development in varied habitats and a microscopic analysis of anatomical changes during growth was made. A description of the developmental morphology in the natural environment was prepared as a compendium





of the information obtained.

### Materials and Methods of Study

During the summer of 1967 over 200 basidiocarps of *G. saepiarium* were collected from many habitats throughout the province of Alberta, Canada. Site characteristics of exposure, elevation and vegetation cover in the vicinity of the substratum were recorded as well as physiognomy and position of the basidiocarp on the wood. A microscope was employed in the field to observe characters which might be liable to desiccation upon removal. Here the particular concern was the presence or absence of oidia. A number of developmental stages in basidiocarp formation were placed immediately in cold storage upon removal from the wood and examined microscopically in a fresh state soon thereafter. Others were preserved in 70% ethanol or FAA (Johannsen, 1940) for later study. The presence of other fungi on the same substratum was also recorded and many of these were collected. Care was taken to include portions of the wood where the fruit bodies of two different species were immediately adjacent to one another.

Fresh collections of basidiocarps were obtained from areas in Mexico; New Mexico, Arizona, Florida, Pennsylvania\* and Wyoming in the United States; and Ontario, Manitoba and British Columbia including Vancouver Island and the Queen Charlotte Islands in Canada. Herbarium specimens of *G. saepiarium* and closely related species from the New York Botanical Garden, Mycological Herbarium at Ottawa, and Cryptogamic Herbarium at the University of Alberta were also examined. Cultures from the mycelium of the fresh specimens were prepared for the cultural study.

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\* Specimens from Mexico, New Mexico, Arizona, Florida and Pennsylvania were provided by T. Erwin.





A study area was established near the University of Alberta so that a long-term record of basidiocarp development under different environmental situations could be made. Four sites, in which *G. saepiarium* was found forming basidiocarps, were chosen on the basis of observable differences in the environment. Basidiocarp development from single primordia was observed in the first three sites, while the development of many primordia as well as preformed basidiocarps was examined in site IV.

Site I was an exposed spruce stump on the steep, upper slope of a cut-bank. No immediate tree canopy was present and ground vegetation was sparse until later in the summer. Direct sunlight was received in the area of basidiocarp formation for a three hour period daily during mid-summer.

Site II was a spruce log lying horizontally along a roadway, sheltered by shrubs and herbaceous plants. No tree canopy was present and little direct sunlight was received on the portion of wood where the basidiocarp developed. Topography was flat but well drained. This site proved to be more xeric than sites III and IV but more mesic than site I.

Site III was a spruce stump sheltered by a spruce canopy during the entire summer. A few shrubs and grasses provided additional cover in late summer. Occasional direct sunlight was received through the canopy during June and July but the site was entirely shaded during the rest of the growing season.

Site IV was a composite site of variously arranged spruce logs, cut in three foot sections. Each log was given a number and its position in the site recorded in a habit sketch, Figure 1, page 24. The position and size of previously formed basidiocarps were recorded so that additional growth and new primordia formed during the study could be noted.



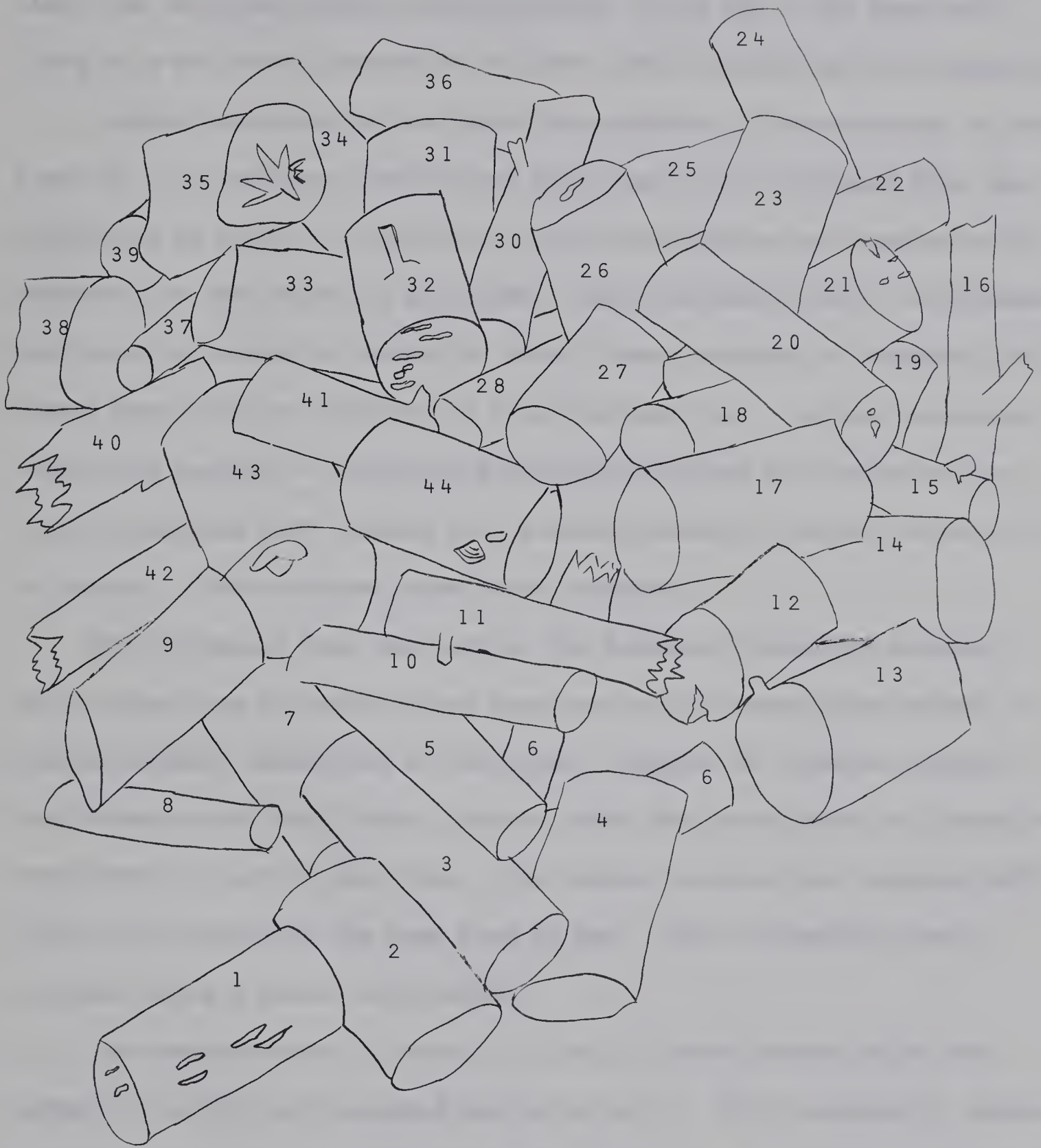


Figure 1. Habit sketch of numbered logs in site III. X 1/20.





There was partial cover provided by a poplar-birch canopy. Direct sunlight was received during various periods of the day. The logs were lying in a sheltered depression but were free from most ground vegetation.

Single basidiocarps developed simultaneously from primordia in sites I and II. An imbricate basidiocarp with four pilei developed from the primordium in site I. Dimensions of the basidiocarps were periodically measured but they were not disturbed. Numerous basidiocarps in different positions and stages of growth in site IV were removed and examined while others were left for observation the following year. During the second summer two logs with basidiocarps which had revived were moved so that the old lamellae were rotated to a position directly opposed to the force of gravity. The continued growth was recorded.

Meteorological data recorded at the Edmonton Industrial Airport\*, three miles from the study sites were used as representative records of general weather conditions at the sites. Records of relative humidity were taken periodically with a Bendix model 566 psychrometer at basidiocarp level in each of the sites. The values obtained were compared with airport data taken at the same time of day. Light intensities were recorded using a Weston light meter.

The basidiocarps in sites I, II and III were removed after two summers of growth and examined microscopically. Their anatomical characteristics were compared with the developmental stages of the basidiocarps which had been collected and examined throughout the study period in site IV. The results were assessed with regard to the environmental conditions under which growth took place.

The anatomical features of the basidiocarp collections were analyzed

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\* Meteorological data supplied by the Meteorological Branch, Department of Transport, Edmonton, Alberta, Canada.





using several techniques. Free-hand sectioning and dissection were found to be most successful in determining basidiocarp structure in the fresh material. The general course of mycelial growth was revealed in embedded material but, because of the length of the mycelial elements, the determination of their origin and final arrangement in any hyphal association was difficult. The methods used in studying the microstructural features of free-hand sections were those outlined by Teixeira (1962). The hyphae were teased apart using variously curved or bent needles which were either honed to a fine point or were blunt and beveled at the edge for cutting and pinning actions. A Leitz micromanipulator was also used but was found to have several disadvantages. The positional relationships of hyphae were not as easily maintained during dissection as when using a high power, stereo-binocular microscope. The tough, flexuous nature of the differentiated mycelium often caused breakage of the glass tools. The speed and efficiency of direct hand manipulation proved to be more satisfactory than the slower but more precise movements achieved by the micromanipulator. The supplementary 2x adaptor lens of the Bausch and Lomb stereomicroscope provided a 60x magnification sufficient for most dissections. One per cent aqueous phloxine was often used to stain hyphal elements containing cytoplasm to distinguish them from differentiated elements lacking cytoplasm.

The mycelium of basidiocarps preserved in 70% ethanol and FAA for more than a week became brittle and therefore difficult to dissect. This material was best observed by embedding it in paraffin, according to the standard procedure described in Johannsen (1940). Freezing, sliding, and rotary microtomes were used to prepare serial sections of different thicknesses, 10-45 $\mu$ , in both preserved and fresh material.



FCF fast-green was used to stain the cytoplasmic hyphae in the prepared slides. A counterstain was unnecessary since structural hyphae were pigmented.

Early vegetative stages of mycelial development in the wood were studied in thin sections prepared by hand or a sliding microtome. Much decayed wood, especially that near the base of the basidiocarp, was best sectioned by embedding it in paraffin with the basidiocarp attached. Staining of hyphae in wood was not necessary since the dense, granular cytoplasm made the vegetative hyphae clearly visible in the tracheids. Various stains were used to detect the presence of hyphae of other fungi in the same substratum. The staining method of Cartwright (1929) was useful in this respect. The freezing microtome was not useful because the hard, resistant nature of the fiber mycelium prevented sectioning without disruption of the spatial arrangement of the mycelium. The thickness of the basidiocarp also prevented uniform freezing.

Because of the three dimensional nature of the basidiocarps, serial sectioning was done in three planes. Confusion in defining the dimensions of the pileus may arise from the fact that basidiocarps are frequently substipitate. *G. saepiarium* characteristically produces a subiculum which varies in thickness from a flat plate closely appressed to the wood to an elongate extension supporting the basidiocarp. If pileus length, as commonly defined, is the horizontal distance between the margin and the base of the basidiocarp at a right angle to the substratum, then a greater width than length can be recorded when including the subiculum. This procedure fails to give a correct picture of the nature of the pileus. In this study "length" is used as a term to denote the greatest distance between the margin and the point closest to the base where







lamellae have formed and at a right angle to the substratum. The term "width" denotes the greatest horizontal extent of the pileus parallel to the substratum.

If the concentric zones of the pileus surface are considered analogous to the annual growth rings of a woody stem and the lamellae to the rays, then a similar terminology for planar sectioning can be used. A tangential section is prepared by sectioning the lamellae at right angles. If the basidiocarp is cut parallel to the lamellae then the section is a radial section. Transverse sections are prepared by cutting in a horizontal plane from the top of the pileus to the bottom.

Serial sectioning provided a unique opportunity to study the changing positional relationships of hyphae in the basidiocarp. Outline drawings of the serial sections mounted on slides were made with the use of a camera lucida. Each drawing was placed on the margin of a series of flexible cards in the same position and order in which the sections were cut. Major areas of hyphal arrangement as seen under the microscope were drawn inside each outline drawing. When the cards were rapidly flipped, the changing image of the drawing reproduced the conformation of the basidiocarp and changes in direction of hyphal associations became evident. This procedure was also helpful in the determination of the exact plane in which the basidiocarp elements were being sectioned, thereby overcoming the disadvantage of the embedding material obscuring the position of the basidiocarp.

Herbarium specimens were microscopically examined by mounting hand-dissected portions of hyphae in 3% KOH. Permanent mounts of all sections were made by passing the material through an ethanol series to 100% xylene. Cover slips were added and sealed with permount, diluted with toluene, and weighted until dry.



Photomicrographs were taken with a Vickers Photomicro 35mm camera fitted with a cadmium sulphide photometer. A Leitz Reflex Bellows Extension plate camera was also used. Illustrations were prepared with the aid of a camera lucida or from film negatives placed in an enlarger and drawn free-hand.

### Environmental Factors and the Physiognomy of the Basidiocarp

Basidiocarps of *G. saepiarium* collected in Alberta were found mainly on dead, fallen, coniferous trees. They developed in the more exposed sites of forests which had been thinned due to fires, seismic lines and access roads. It is possible that fallen trees may be decayed by *G. saepiarium* in dark, damp woods, but it was rarely found fruiting there. Although collections were made in spruce-bogs and coniferous stands in aspen-parkland, basidiocarps were rare or absent in pure hardwood stands. Basidiocarps were found occasionally on poplar, aspen and alder, but were never found on birch, although it was often present. No consistent difference in the morphology of the basidiocarps was found that could be attributed to species difference of the substratum. Contrary to previous reports (Cartwright and Findlay, 1958) basidiocarps occasionally developed on small twigs and branches. Severe environmental conditions may have accounted for the absence of basidiocarps on krummholtz vegetation above timberline. They were observed in the marginal zones of tree cover at timberline.

In the more mesic sites, basidiocarps of *Fomes cajanderii* Karst, *Fomes pini* (Thore ex Pers.) Lloyd, and *Hirschioporus abietinum* (Dicks. ex Fr.) Donk were often found on the same substratum with those of *G. saepiarium*. In several instances the basidiocarps of *H. abietinum* were





almost in contact with those of *G. saepiarium*. Microscopic examination of the wood immediately below adjacent basidiocarps revealed mycelium of both species occupying the same tracheid. The hyphae of these species were quite different and their interaction was easy to follow. No zone lines were evident where two species were adjacent to one another. The wood was often greatly darkened and decayed in the vicinity of basidiocarp attachment. Dark-walled hyphae and spores of dematiaceous imperfects were commonly observed in the surface layers of tracheids beneath basidiocarps forming on cross-sectioned ends of logs. They were also observed to be incorporated in the mycelium of the basidiocarp at its base.

In xeric sites, *Gloeophyllum (Trametes) odoratum* (Wulf.) Imazeki was frequently present on the same wood substratum with *G. saepiarium* as were species of *Coniophora* DC. ex Mérat and *Stereum* Pers. ex S.F. Gray. Instances in which *G. saepiarium* was the only macroscopically observable fungus were more frequent in the xeric sites. The presence of other fungi had no apparent effect on the morphology of the basidiocarps.

Four general categories of variability in physiognomy of the basidiocarps were observed in the natural environment. They were: variability in form due to position on the substratum; variability in form due to some other factor; differences in hymenial conformation; modifications of the abhymenial surface. In his monograph of the genus *Lenzites*, R. Falck (1909) presented detailed descriptions and classifications of practically every morphological variation within the first three categories that I had the occasion to observe. A modified resume of his classification is given in Appendix I.

The shape of the basidiocarp is either circular or semicircular and the position in which it forms influences the expression of individual





symmetry. This situation is not unique for this species. Basidiocarps of many other polypores have similar shapes and respond in a similar manner to position on the substratum. However, the facility with which individual basidiocarps fuse to form compound structures and the manner in which additional mycelium is added to existing basidiocarps is striking in the genus *Gloeophyllum* and perhaps most pronounced in *G. saepiarium*. As Falck has indicated, some of the variability appears to be genetically controlled and often independent of environmental influence. For example, hymenial configuration can vary regardless of position on the substratum. Also basidiocarps of diverse morphology can be found in similar positions and in similar environments as was the case in site IV. An effused-reflexed basidiocarp was found on the end of log 20, Figure 7, page 74, and a sessile dimidiate basidiocarp was found on the end of log 44, Figure 8, page 74. Both logs were in similar positions, Figure 1, page 24. The nature of internal control and the modifications in the site due to environmental conditions of the first three categories of variability will be discussed later in the description of basidiocarp development.

It was evident that external factors were responsible for some of the variability found in the fourth category. The abhymenial surface of the pileus was found to be different in separate geographical areas and in local habitats where there was a wide range of environmental conditions.

The majority of the specimens examined from Mexico, the United States and Canada were essentially similar in having a variously colored, concentrically zoned surface. The zones varied from slightly indented to deeply sulcate. The surface between zone margins was strigose-tomentose and became hirsute-tomentose at the margins. The erect nature



of agglutinated, skeletal hyphae was dramatic in several specimens, some groups of hyphae projecting to a height of 4 mm.

Collections from the islands of British Columbia were conspicuously different from those mentioned above in lacking the sulcate zonations and possessing a fibrillose tomentum. The skeletal hyphae were aggregated in a parallel fashion, were rarely erect and ended at the zone margins. The margin of the pileus was serrate or appendiculate due to the extension of the hyphal aggregates.

Basidiocarps on driftwood along waterways were examples of variation within local habitats. They were small, rarely more than 2 cm long, with a compact, dark-colored, uniformly hirsute-tomentose surface. The zonations were narrow, indicating the slow growth that took place under conditions of high insolation and rapid evaporation, but with periodic flooding or rainfall to allow some growth.

One other form of the abhymenial surface found in several basidiocarps should be mentioned since it was not typical of any particular geographic area or locality. The surface was finely tomentose, almost velutinous, becoming glabrous in age. Zonations were nearly smooth and evident because of color differences. The skeletal hyphae which composed the tomentum were only moderately thick-walled and rarely agglutinated. They were aggregated in narrow flexible strands in which the individual hyphae were often twisted and collapsed.

A summary of the characters observed in representative samples of all basidiocarp collections as well as herbarium material is presented in Table I, page 33. Characters, other than the tomentum, found to differ considerably in the basidiocarps examined were: presence and features of the cystidia, depth of the context, diameter and wall







Table I. Summary of range of variability in *Gloeophyllum saepiarium* in the natural environment.

Basidio-carp	Annual or reviving several years, never horizontally stratified; resupinate or pileate (apileate under conditions of high humidity and low light intensity).
Pileus	Sessile, substipitate or effused-reflexed; dimidiate, flabel-liform, usually solitary but occasionally laterally connate or imbricate; applanate, conchate, or infundibuliform; concentrically zonate, the zonations smooth to deeply sulcate and variously colored*, burnt umber (MP-15A12), golden chestnut (MP-14A9), gold brown (MP-14F12), sandy beige (MP-14A3), slate gray (MP-14A1); margin acute or obtuse, usually entire but occasionally serrate or appendiculate, the contour even, undulate or lobate, white, yellow or orange-brown when fresh becoming dark brown or black with age; dimensions variable, 0.5-10.0 cm wide (20 cm or more when laterally connate), 0.5-5.0 (9.0) cm long.
Tomentum	Hirsute-tomentose throughout or only near zone margins with intervals between the zones strigose-tomentose, zones variable in color, rarely concolorous.
Types	
(1)	
(2)	Fibrillose-tomentose, striate in direction of growth, zonation marked by ends of agglutinated fibers which produce a serrate or appendiculate margin.
(3)	Finely strigose-tomentose, occasionally velutinate to glabrous between zones, zones smooth or only slightly sulcate near the margin and marked by color differences.
Context	Coriaceous, flexible when fresh becoming rigid on drying, 0.5-8.0 mm thick; concolorous or with distinct zonations revealed by sectioning at right angles to the direction of growth, rust yellow (MP-13J9 or MP-13I8), to gold brown (MP-14F12), or dark brown (MP-14A12).
Dissepiments	Lamellate with occasional cross septations, labyrinthiform, rarely uniformly daedaloid, never uniformly poroid but occasionally poroid near the base.
Lamellae	Parallel or radiate; entire or occasionally laciniate or toothed, straight or sinuous; 12-22(26) per centimeter, 1.0-8.5 mm deep, thick at base thinning to obtuse or acute margin, occasionally 0.6 mm thick, rarely membranaceous.



Table I. (continued)

Hyphal Systems	Generative--1.5-3.6 (4.8) $\mu$ diameter, nodose-septate, occasionally thick walled and light yellow in color.
(1)	
(2)	Skeletal--2.0-3.7 (5.2) $\mu$ diameter, walls colored, yellow to buff (MP-11K7), up to 1.4 $\mu$ thick but variable, lumen rarely occluded, aseptate, or with convex plasmatic septa, often collapsed or twisted in the context.
(3)	Binding--1.5-2.5 (3.2) $\mu$ diameter, hyaline to light yellow in aged specimens, variable branching but usually long 15-35 $\mu$ (2mm), scattered or rare, found most frequently near the base of the pileus or in the subiculum.
Cystidia	Rare to numerous, hyaline, smooth or capitate, rarely encrusted, 3.2-4.5 $\mu$ even with or projecting 7-15 $\mu$ beyond hymenial layer, cylindrical to fusiform, the narrow tips often quite long, 6-8 $\mu$ , and can be confused with tips of skeletal hyphae which grow through the hymenium.
Basidia	Clavate, 18-34 x 3.1-5.2 $\mu$ , occasionally abortive or elongating at the tip to 50 $\mu$ .
Spores	Hyaline, or cream to pale yellow in mass, ellipsoid cylindric when young, cylindric to slightly allantoid when mature, 7-11 (13) x 2.5-3.8 $\mu$ .

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\* Colors are according to Maerz and Paul (1932), MP.





thickness of skeletal hyphae, presence of binding hyphae and numbers of lamellae per centimeter. The type of skeletal hyphae and their orientation is directly associated with the appearance of the abhymenial surface as well as the appearance of zonation within the context. The abundance of binding hyphae was variable in different parts of the same basidiocarp and they were absent in several instances. Since these characters, including lamellae and their hymenial constituents, are products of hyphal differentiation and orientation, their diversity will be considered in the discussion of basidiocarp development. Basidiocarps which were macroscopically different did not possess any unique microstructural features or combinations of characters to indicate that they might represent another species. One lamellate collection from Graham Island, B. C., was both macroscopically and microscopically distinct from the other specimens and is presently regarded as a new variety or species of *Gloeophyllum*. Additional study is needed to verify this diagnosis.

The inherent difficulty in the taxonomic separation of *G. saepiarium* from related species arises from the fact that there are no characteristics unique for any given species. The overlap of characters within the genus and in allied genera is depicted in Table II, page 36\*. It is only in the recognition of a complex of characters that the distinctive nature of each species can be macroscopically and microscopically determined. The importance of "character-complexes" can be illustrated at the generic

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\* The descriptions presented are composites of those previously published and my own observations, where noted. *G. Berkeleyi* and *D. elegans*: Murrill (1908) and Fidalgo and Fidalgo (1966). *G. trabeum*, *G. odoratum*, *L. betulina* : Overholtz (1953) and personal observations. *G. abietinum*: Macrae and Aoshima (1966) and personal observations. *G. saepiarium*: personal observations. (No European collections of *G. saepiarium* were examined.) Colors are according to Ridgway (1912), R, and Maerz and Paul (1932), MP.



Table II. Basidiocarp descriptions of members of the genus  
*Gloeophyllum* and related species.

Species	Pileus Description	Hymenium	Context		Hyphal Systems			Cystidia	Basidiospores	Decay	KOH Reaction
			Thick- ness	Color	Gener- ative	Skele- tal	Bind- ing				
<i>Daedalea elegans</i> (Spreng ex Fr.) Syn.: <i>Lenzites repanda</i> <i>L. palisoti</i> <i>L. polita</i> <i>D. ambigua</i>	sessile to short stipitate; dimidiate, or flabelliform; corky, flexible to rigid; azonate to zonate at the margin; margin concolorous, entire; tomentum finely velvety to glabrous (shiny), white, gray to black, MP-11C2 (ecru beige)	daedaloid to lamellate, 22-26/cm	2-14 mm	milk white to cream (MP-9D2)	1.5-4 $\mu$	3-5 $\mu$	1.5-2.5 $\mu$ hyphal complexes	none pseudopary-physes	elliptic to cylindric 5-6.5 x 2-3 $\mu$	white rot	slightly yellowing
<i>Lenzites betulina</i> (L. ex Fr.) Fr. Syn.: <i>D. betulina</i>	sessile or effused-reflexed; dimidiate, or flabelliform; coriaceous, flexible to rigid when dry; zonate, zones sulcate and multicolored, white, avellaneous, olivaceous; margin entire, undulate or lobate; tomentum hirsute to tomentose	lamellate, or with occasional cross septations, 8-15/cm	0.5-3 mm	white to cream brown on drying	3-6 $\mu$	4-7 $\mu$	1.8-3.5 $\mu$ hyphal complexes	hyaline 24-40 x 4-7 $\mu$ projecting 10-20 $\mu$	subglobose to short cylindric 4-6.5 x 2.5-3.5 $\mu$	white rot	negative
<i>Gloeophyllum striatum</i> (Sw. ex Fr.) Murr. Syn.: <i>L. striata</i>	sessile, rarely substipitate; dimidiate or flabelliform; flexible, coriaceous; marginally zonate or azonate; margin thin, entire; tomentum finely tomentose to velutinous, brown to dark brown, MP-15C8, 13B2, 11C3	uniformly lamellate, 14-26/cm	0.5-3.5 mm	medium brown (MP-15C8) to dark brown (MP-15H10)	2-4 $\mu$	3-6 $\mu$	1.5-3.5 $\mu$ much branched	hyaline to yellow, fusiform, encrusted 20-36 x 3.5-5.5 $\mu$ , projecting 3-6 $\mu$	cylindric to cylindric ellipsoid 6-11x2-4 $\mu$	brown rot	black
<i>Gloeophyllum abietinum</i> (Bull. ex Fr.) Karst. Syn.: <i>L. abietinus</i>	sessile, substipitate or effused-reflexed; dimidiate, imbricate or laterally connate; coriaceous; zonate; margin acute or slightly thickened, entire, concolorous; tomentum finely strigose-tomentose or velutinous becoming glabrous, snuff brown to buckthorn brown, R-XXIX and XV	usually lamellate but occasionally daedaloid to labyrinthiform, 13-22/cm	1-2.5 mm	snuff brown, blister brown, Saccardo's umber (RXXIX)	1.9-4.8 $\mu$	2.2-5.7 $\mu$	2.2-3.1 $\mu$ occasional sparsely branched	numerous, hyaline or colored, fusiform, encrusted 29-62x4.8 - 7.5 $\mu$ projecting 14-18 $\mu$	cylindric to slightly allantoid 8-12.5 x 2.8-4 $\mu$	brown rot	black
<i>Gloeophyllum saepiarium</i> (Wulf. ex Fr.) Karst. Syn.: <i>L. saepiaria</i> <i>L. abietinella</i> <i>L. hirsutum</i>	sessile, substipitate or effused-reflexed; dimidiate, imbricate or laterally connate; coriaceous; zonate, often deeply sulcate; margin thin to obtuse, entire or serrate, brightly colored when young; tomentum hirsute, strigose-tomentose or fibrillose, rarely velutinate, variously colored, sandy beige (MP-14A3) to burnt umber (MP-13A12)	usually lamellate occasionally daedaloid or labyrinthiform, poroid in older portions but never uniformly so, 12-22 (26)/cm	0.5-8 mm	gold brown (MP-14F12)auburn (RII) antique brown (RIII) prouts brown (RXV)	1.5-3.6 (4.8) $\mu$	2-3.7 (5.2) $\mu$	1.5-2.5 (3.2) $\mu$ occasional sparsely branched	rare to numerous, hyaline, cylindric to fusiform 20-55 x 2.8-4.5 $\mu$ , projecting, 5-17 $\mu$	cylindric 7-11 (12) x 2.2-3.8 $\mu$	brown rot	black
<i>Gloeophyllum trabeum</i> (Pers. ex Fr.) Murr. Syn.: <i>Trametes trabea</i> <i>Coriolopsis trabea</i> <i>Lenzites trabea</i>	sessile, substipitate or effused-reflexed; dimidiate, laterally connate; coriaceous; lightly zonate or azonate; margin thin to slightly thickened, entire, concolorous; compactly tomentose, largely hirsute, becoming glabrous; dull to medium brown becoming black or gray with age	daedaloid occasionally sublamellate 26-38/cm	1-4 mm	snuff brown, tawny olive (R-XXIX), buckthorn brown (R-XV)	2-4 $\mu$	3-6 $\mu$	1.8-3.1 $\mu$ occasional to rare sparsely branched	rare, hyaline to light yellow, cylindric, 20-40 x 3-5 $\mu$ , projecting 4 $\mu$	cylindric to cylindric-ellipsoid, 7-10 x 2.5-4 $\mu$	brown rot	black
<i>Gloeophyllum Berkeleyi</i> (Sacc.) Murr. Syn.: <i>Daedalea Berkeleyi</i>	sessile; dimidiate, imbricate or laterally connate; corky or coriaceous, zonate; margin thick or obtuse, entire; tomentum compactly tomentose to glabrescent, medium brown to black, MP-14D7, 15A2, 8C8, 15A6	daedaloid to poroid or somewhat lamellate pores 8-12/cm gills 10-20/cm	2-15 mm	cocoa (MP-7E12), brown (MP-7H12)	2-4 $\mu$	2.5-7 $\mu$	2-4 $\mu$ rare	none	cylindric 7-13(14) x 3-4.5 $\mu$	brown rot	black
<i>Gloeophyllum odoratum</i> (Wulf ex Fr.) Imazeki Syn.: <i>Fomes odorata</i> <i>Osmoporus odorata</i> <i>Trametes americana</i>	sessile or effused-reflexed; applanate, often thick and triangular in x.s.; coriaceous rigid, zonate but rarely sulcate, margin obtuse or thick tomentum compactly tomentose or fibrillose becoming agglutinated into a seared surface, rusty yellow or brown weathering to whitish gray	poroid to daedaloid, never lamellate 2-3 pores/mm	2-20 mm	beige to gold brown (MP-14F12)	1.8-3.2 $\mu$	2.5-5(6) $\mu$	1.8-2.8 $\mu$ occasional	none	cylindric-cylindric-ellipsoid 9-12x3-4.2 $\mu$	brown rot	black



level in the separation of *Gloeophyllum* from the original genus, *Lenzites* Pers. ex Fr.

The genus *Gloeophyllum* was erected by Karsten (1886) to separate the coriaceous, lenzitoid polypores with brown basidiocarps, typified by *G. saepiarium*, from those with white or light-brown basidiocarps, typified by *Lenzites betulina* (L. ex Fr.) Fries. Murrill (1908) considered hymenial configuration of primary importance in establishing generic limits and grouped the daedaloid and lamellate species in the tribe Daedaleae. He retained Karsten's two genera and added *Daedalea* (Pers. ex Fr.) Fries and *Cerrena* Mich. ex S. F. Gray. The close relationship of *Daedalea* to *Lenzites* as compared to *Gloeophyllum* can be seen by comparing the definitive character-complexes of each group. The *Lenzites-Daedalea* listing in Table II, page 36, is representative of a number of species having the following characters in common: white or light-brown context failing to turn black when moistened with KOH, binding hyphae forming hyphal complexes, spores subglobose to short cylindric,  $4.5-7.0 \times 1.5-4.0\mu$  (except *D. confragosa*,  $7.0-9.0 \times 2.0-3.0\mu$ ), white rot decay (except *D. quericina*), absence of cystidia but with simple or bifurcate pseudoparaphyses (except *D. unicolor* and *L. betulina*), variable hymenial conformation, usually lamellate or daedaloid but never poroid.

In contrast to the above species, members of *Gloeophyllum* exhibit the following character-complex: brown or dark-brown context turning black in KOH, spores cylindric-ellipsoid to cylindric, large,  $7.0-14.0 \times 2.5-4.5\mu$ , brown rot decay, cystidia abundant to lacking, no paraphyses, binding hyphae present but not as hyphal complexes, hymenial conformation lamellate, daedaloid or poroid, occasionally irpicoid.

The segregation of individual species of *Gloeophyllum* into







monotypic genera or placement in seemingly unrelated genera, particularly in the case of *G. odoratum* and *G. trabeum* (see synonymy in Table II), seems unjustified when character-complexes provide good evidence for their retention. *G. saepiarium* and *G. odoratum* exhibit enough similarity in their morphological characteristics to be confused occasionally with one another. Yet these two species have been placed in different genera. Using several features of the character-complex it is possible to point out the homogeneity of characters which confine them to the genus *Gloeophyllum* as well as their interrelationships with other members of the genus.

The species of *Gloeophyllum* are listed in Table II, page 36, in the order of decreasing similarity when considering the following characters:

1. Abhymenial surface changing from hirsute-tomentose to compactly tomentose or fibrillose, appearing encrusted but anoderm.
2. Pilei which are sessile, effused-reflexed or substipitate changing to sessile or effused-reflexed, never substipitate. Effused-reflexed pilei are triangular in cross section in the latter and are L-shaped in the former.
3. A predominantly lamellate hymenial configuration changing to one that is predominantly poroid.
4. A thin margin changing to a thick or obtuse margin in mature specimens.
5. Cystidia abundant, fusiform, and colored changing to cystidia inconspicuous or absent.

The almost uniform transition of these characters from one species to



another is striking. To what extent each character is interrelated with the others is not known since the range of variation and developmental sequence was not studied for species other than *G. saepiarium*.

Hyphal systems were similar for species in *Gloeophyllum*. The absence of hyphal complexes in the binding hyphae was helpful in separation at the generic level. Spore characters were different in the two genera, *Gloeophyllum* and *Lenzites*. In general, spore characters were not distinctive for the species of *Gloeophyllum*, although there was some difference in the range of spore size. Color, although helpful to a person well acquainted with its manifestations in these species, is often misleading for several reasons:

1. Color changes with the method of preserving the specimen. It usually darkens if the basidiocarp is left to dry slowly. Dried specimens lose color intensity.
2. Color varies with age, the younger specimens showing the more characteristic hues, the older specimens turning to similar browns, blacks and grays. The pigments in the *Gloeophyllum* species are water soluble and are leached from specimens in areas of high precipitation.
3. Color differences are confusing when several are present at the same time in different zones. Color is also masked by differences in texture of the tomentum.

The coloration of the context is much more helpful than the pileus surface since the context is less exposed to environmental conditions.





## Development of the Basidiocarp

### A. Macroscopic features

The morphological changes which occurred during the growth of a number of basidiocarps in different environments were periodically observed in the study area for two summers. The macroscopic changes during basidiocarp development in sites I, II and III for the summer of 1967 are recorded in Table III, page 42. A summary of the weather conditions preceding the dates of observation is also included.

Small basidiocarps had formed the previous summer in sites I and II. A primordium had formed in site III. Renewed growth occurred simultaneously in all three sites early in July. The growth increment was very slight and confined to the marginal areas of the basidiocarps in sites I and II while the entire surface of the primordium in site III was covered with a thin felt of new hyphae. Normal weather conditions were recorded for May. June and July had normal temperatures but below normal precipitation. The first measurable growth increase was noted on August 1. A second and larger increase was observed on August 13. Both growth increments were preceded by periods of precipitation and high temperatures. In sites I and II the growth was again restricted to the margins of the pilei and the lower portions of the lamellae. The cessation of growth was marked by a yellowing of the new mycelium and the formation of lamellae on the undersurface of the new margin. Lamellae were noted for the first time on the primordium in site III on August 15. They had formed from the previous growth on the margin of the primordium, now referred to as a basidiocarp. Two additional growth increments in sites I and II were recorded later in the summer during a period of low





Table III. Basidiocarp growth and growing conditions during the summer of 1967 in sites I, II, and III.

Date of Observa- tion	Summary of Weather Pre- ceding Observation			Growth Record		
	Rain- fall Inches	Temp. °F (Mean)	Weather	Site I	Site II	Site III
May	1.58 (normal 1.83)	51.0	Sunny cool showers	Basidiocarp No growth	Basidiocarp No growth	Primordium No growth
July 2	June 1.72 (normal 3.15)	June 57.7	Partly cloudy Showers	New hyphae at margin	New hyphae at margin	New hyphae at margin
July 15	0.38	64.5	Sunny Warm	No growth	No growth	No growth
July 26	1.75	67.0	Cloudy Warm Thunder- showers	No growth	No growth	No growth
August 1	0.27	66.0	Warm sunny	Marginal growth 1.5 mm diam	Marginal growth 1.5 mm diam	New hyphae on margin and surface
August 13	2.56	66.4	Sunny very warm thunder- showers	New growth zone on margin 5 mm diam	New growth zone on margin 3 mm diam	New hyphae 2 mm deep on surface. no zones.
August 15	0.12	71.5	Sunny hot thunder- showers	Previous zone orange- brown. Lamellae have formed	Previous zone orange- brown. Lamellae have formed	Marginal lamellae have formed. Basidiocarp light colored.
August 26	0.17	67.2	Sunny very warm	New margin- al zone, 1 mm diam Desiccation and shrink- age of hyphae	New margin- al zone, 2 mm diam Desiccation and shrink- age of hyphae	New hyphae on surface but no zones. Surface is uneven and furrowed.
September 2	0.10	68.1	Sunny hot windy	New zone 1.5 mm diam but unevenly deposited.	New zone 1 mm diam brown and drying	No growth Color becom- ing brown
September 14	none	61.6	Sunny Warm windy	No growth	No growth	Basidiocarp moist No growth
September 26	0.03	63.9	Sunny Warm windy	No growth	No growth	No growth





precipitation and high temperature. The last addition was irregularly deposited and the surface hyphae were dark-colored and desiccated. All growth increments during the summer were evident as distinct zones on the pileus surface except those that formed on the basidiocarp in site III.

Formation of new zones terminated in September when the relative humidity of the sites was low. In Figure 2, page 44, the relative humidity of the sites I, II and III taken at each observation is compared to that recorded at the Edmonton Industrial Airport at the same time of day. Unfortunately the data for site IV was lost. The relative humidity values of each site confirmed the observable differences in the sites attributed to moisture conditions. The latter part of the summer of 1967 was windy, hot and dry and the higher relative humidity usually maintained by the surrounding vegetation dropped and approached more closely the values of the airport.

The periodic changes in basidiocarp dimensions at the end of the first summer are illustrated in Figure 34, page 88. The basidiocarps increased in size but some of the measured gain was lost due to shrinkage during dry periods. Although site III was the more mesic site, the basidiocarp that formed there made no exceptional gains in marginal dimensions. Its physiognomy changed from a relatively uniform mound of dense mycelium to a basidiocarp with marginal lamellae and an irregular, furrowed surface lacking distinct zonation.

The amount of basidiocarp growth in terms of increase in marginal diameter in site IV was slightly greater than that in the other three sites. The average total increase in marginal diameter for 14 measured basidiocarps was 7.5 mm while the maximum increase was 10.0 mm. Renewed growth is seen as a definite zone on the basidiocarp illustrated in





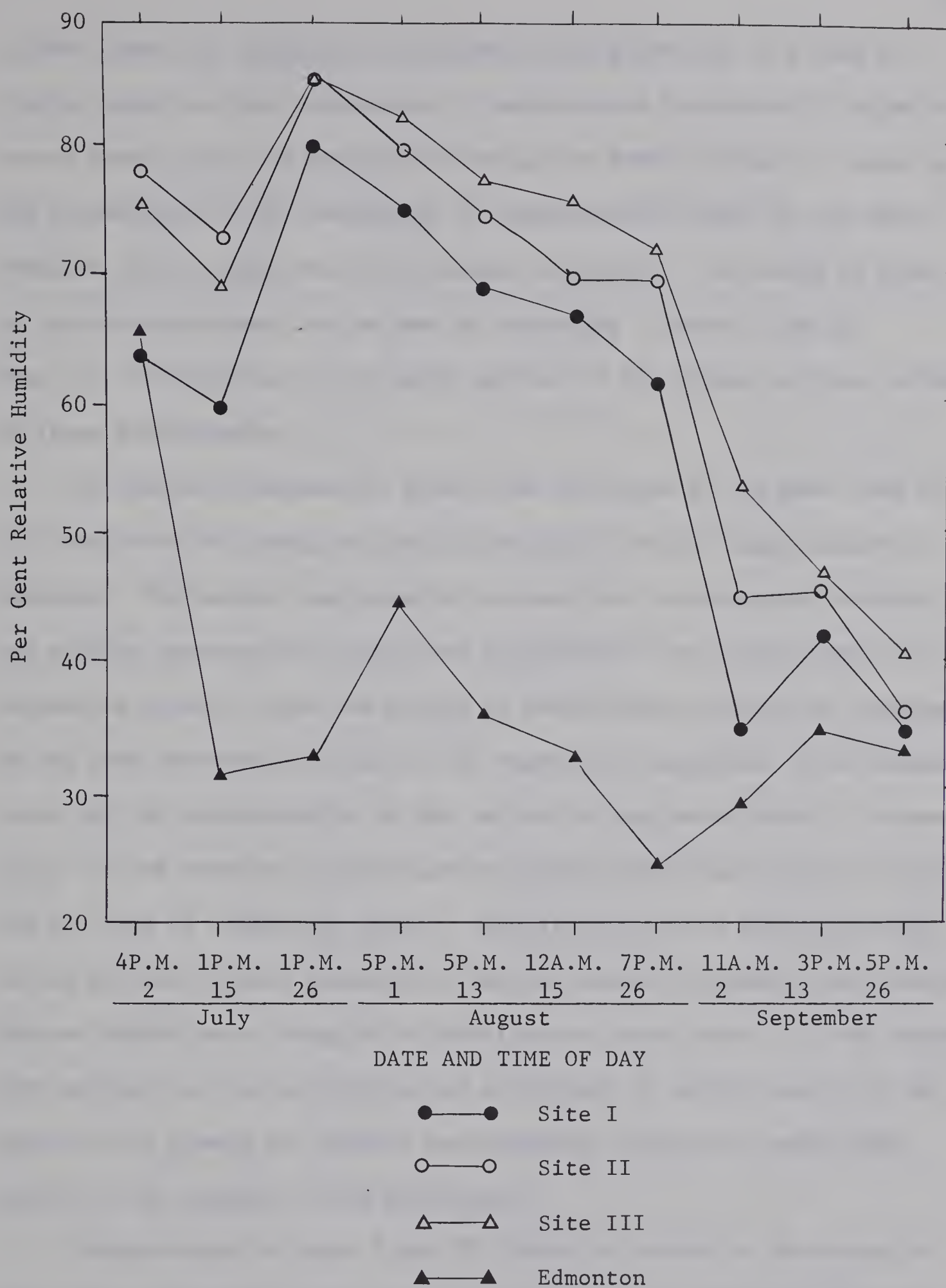


Figure 2. Per cent relative humidity in sites I, II, III, and at the Edmonton Industrial Airport during the summer of 1967. Data represent single values taken at the same time of day in each locality.



Figure 5, page 74. Individual increments during the year are seen as lighter zones on this basidiocarp. Basidiocarps occasionally failed to revive evenly over the established margin as seen in Figure 6, page 74. The variability in the morphology of basidiocarps formed in the same position on the substratum was evident in site IV. Variation in type of basidiocarp formed can be seen by comparing Figures 7 and 8, page 74. Differences in the upper surface of the pileus are also evident on these basidiocarps.

In general, basidiocarp growth was initiated at the same time in all four sites following periods of precipitation and high relative humidity. The marked time interval between the occurrence of rainfall and visible reproductive growth was attributed to an initial period of vegetative growth, since the growth of basidiocarp mycelium is dependent on the food reserves provided by the vegetative mycelium. Cool temperatures and low precipitation in May and early June were probably responsible for the absence of reproductive growth during that period by slowing the rate of vegetative growth. Basidiocarp growth which occurred during periods of high temperature and low relative humidity was limited. The new hyphae were irregularly deposited and desiccated. It was supposed that moisture in the substratum was sufficient to allow vegetative and reproductive growth but adverse environmental conditions restricted growth on the outside of the substratum.

Basidiocarps in sites I and II failed to revive in the summer of 1968. The reason for this is not known but it is possible that the dry conditions in the latter portion of the summer, 1967, caused their death. Again basidiocarp growth in site III was recorded along with weather data from the airport in Table IV, page 46. The summer began with the driest





Table IV. Basidiocarp growth and growing conditions during the summer of 1968 in sites III and IV.

Date of Observa- tion	Summary of Weather Preceding Observation			Growth Record	
	Rain- fall Inches	Temp. °F (mean)	Weather day- time	Site III	Site IV Log 23
May 23	0.07	52.1	Cool Windy	No growth	No growth
June 18	1.39	58.2	Cloudy Warm Showers	No growth	New primordium
June 26	0.28	60.6	Partly cloudy Warm	No growth	1 mm increase on surface. Hyphae yellow.
July 2	0.50	55.1	Partly cloudy Very warm Showers	No growth	No growth. Pre- vious growth brown.
July 12	0.23	69.5	Sunny Hot winds gusty	New hyphae on margin of basidiocarp	No growth
July 27	1.97	59.7	Partly cloudy Thunder- showers Warm	Marginal growth zone. 6.5 mm diam. White	New primordium Old primordium mostly desic- cated.
August 7	2.33	59.6	Cloudy Warm Thunder- showers	Previous growth increased to 8.5 mm diam Hyphae yellow.	Slight growth on surface of new primodium. Old primordium dead.
August 18	none	57.6	Partly cloudy Cool	New marginal zone 8.0 mm diam. Four pilei evident.	New marginal zone with lamellae. 6.0 mm diam
August 29	1.32	58.9	Cloudy Showers Cool nights	New marginal zone 4.5 mm diam. Old zones becoming dark brown, stri- gose-tomentose	New marginal zone 9.5 mm diam. Old zone light brown.
September 14	0.68	51.9	Cloudy Cool Showers	Small zone on up- per pileus 0.5- 1.0 mm diam. Zo- nation very dis- tinct on all pil- ei. Tomentum be- coming differen- tiated.	No growth. Surface becoming strigose- tomentose.
September 27	.66	42.6	Cloudy Very cool Showers	No growth	No growth





May on record. The basidiocarp in site III was observed during May and June but no growth was evident, Figure 9, page 76. The first half of June was warm and cloudy with occasional rain showers and by June 18, new growth was evident on revived basidiocarps in site IV. A new primordium on log 23 of site IV, Figure 12, page 78, was observed throughout the summer and changes in its development recorded in Table IV, page 46.

The first part of July was very warm and dry. Growth had stopped by July 12. Heavy precipitation and moderate temperatures between July 19 and July 23 induced major growth of basidiocarps in sites III and IV. Near the end of the growing period, on July 27, the conformation of the new margin on the basidiocarp in site III indicated that several individual pilei were forming from the original, elongate primordium, Figure 10, page 76. In site IV the primordium had revived but most of the surface hyphae were desiccated. There was a new light colored primordium growing beside it as illustrated in Figure 13, page 78.

Two heavy thundershowers on August 5 and 6 preceded renewed growth on August 18. A total growth increase of 8 mm at the margin of the basidiocarps in site III occurred between August 9 and August 18. With the formation of lamellae, the new primordium in site IV was considered a basidiocarp. The older primordium beside it was apparently no longer living.

Frequent periods of precipitation preceded a third growth increment recorded on August 29. The new marginal addition on the basidiocarp in site IV is illustrated in Figure 14, page 78. Most growth stopped in site IV during the first part of September but some new zones were noted on several basidiocarps in both sites before they reached their final dimensions on September 14. The final morphology of the basidiocarp in



site III is illustrated in Figure 11, page 76, and a summary of the periodic changes in basidiocarp dimensions is presented in Figure 35, page 90.

The relative humidity values of site III, when compared to airport records, Figure 3, page 49, show that a high relative humidity was maintained in the site except during the hot, dry weather of early July. No basidiocarp growth occurred during this period and adverse environmental conditions may have been responsible for the death of the original primordium on log 23 in site IV. No precipitation occurred between August 7 and August 18. The new growth zone that formed during the last seven days of this period appears to have been formed as a result of vegetative growth stimulated by heavy precipitation that occurred on August 5 and 6. Relative humidity and temperatures were high during the formation of the growth zone.

An imbricate basidiocarp with four pilei developed in site III during 1968 with an average increase in marginal diameter of 2.15 cm as compared to a 0.45 cm increase in 1967. The amount of basidiocarp growth in site IV during 1968 was considerable. The basidiocarp illustrated in Figure 15, page 80, developed from a primordium which was initiated in June and attained a final size of 5.5 x 4.5 cm at the end of September. In comparison, the maximum growth for any basidiocarp observed in site IV during 1967 was 1.0 cm. The difference in basidiocarp development can be, in part, attributed to the differences in moisture conditions between the two years. In 1968, 10.13 inches of precipitation was recorded, 1.85 inches more than occurred in 1967. In 1967 most of the rainfall came in the form of thundershowers with intervals of warm dry weather. In 1968 the moisture came during







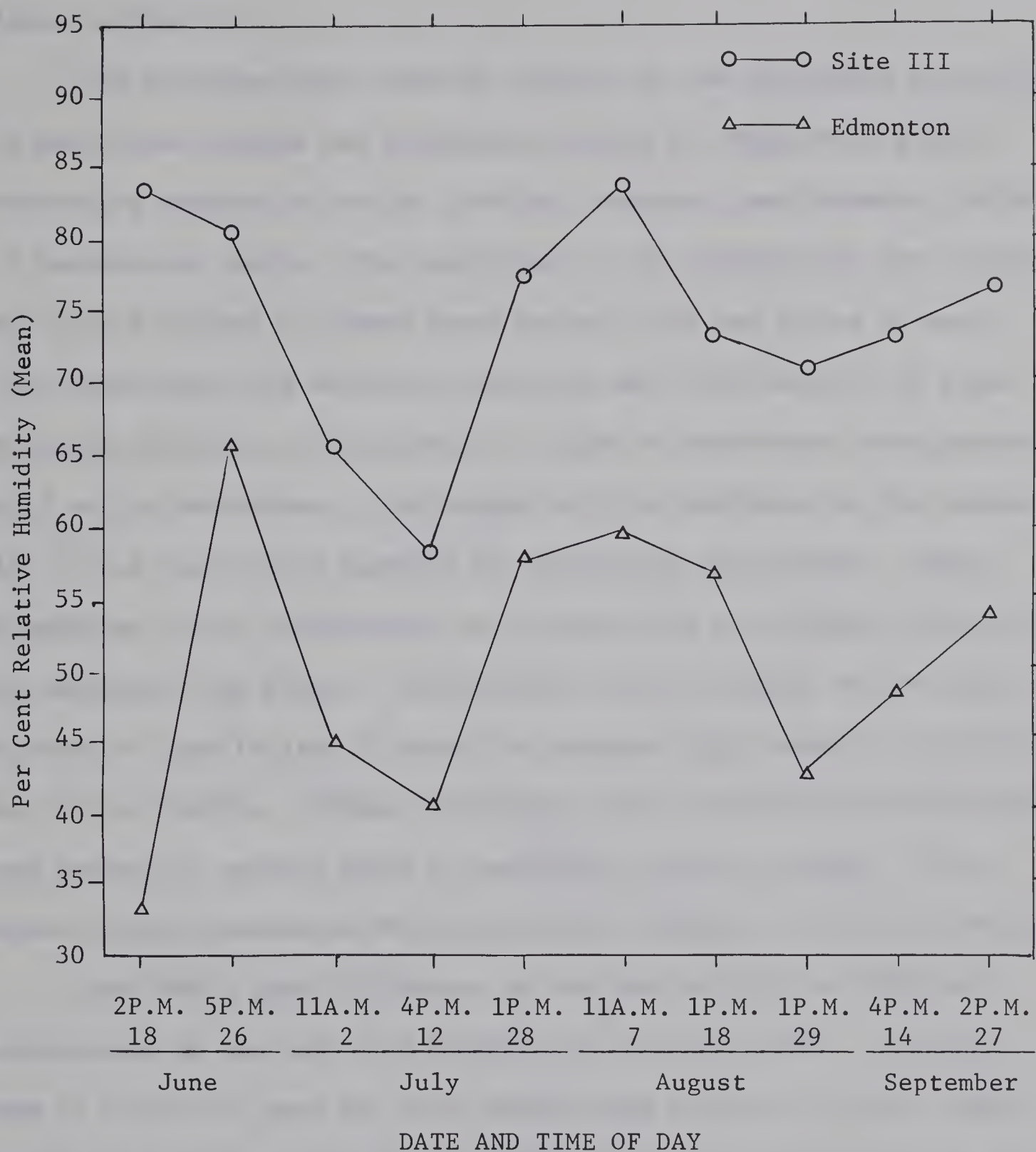


Figure 3. Per cent relative humidity in site III and Edmonton Industrial Airport during the summer of 1968.



extended periods of cool, cloudy weather as light to heavy showers and thundershowers. This difference is reflected in the comparison of the monthly average mean relative humidities of both years, presented in Figure 4, page 51.

With sustained high relative humidity in the atmosphere surrounding the substratum surface and available moisture for vegetative growth, temperature appeared to be the limiting, external, environmental factor for basidiocarp growth. The morphology of the basidiocarps was altered when they developed in shaded areas beneath bark and pieces of wood. Since temperature and moisture conditions were also modified in these sheltered positions, the influence of light on basidiocarp development could not be determined. This aspect will be considered in the discussion of the microscopic features of basidiocarp development. Light intensities varied considerably in the same site at different times of day throughout the summer. Basidiocarps formed normally on the undersurfaces of logs in site IV where the maximum light intensity recorded was 15 foot-candles. Normal development also occurred when basidiocarps were exposed to several hours of continuous, direct sunlight. They became visibly desiccated when the relative humidity of the site was low.

There was a great difference in the revivability of different basidiocarps on the same logs within site IV during 1968. As can be seen in Figure 16, page 80, some basidiocarps failed to revive, others showed only slight growth on the margins of the old pileus and lamellae. The older portions of the lamellae often failed to revive even on basidiocarps which demonstrated vigorous new growth at the margins. Growth zones were variable in size on the same basidiocarp, Figure 35, page 90, and on individual basidiocarps growing side by side, Figure 17, page 80.



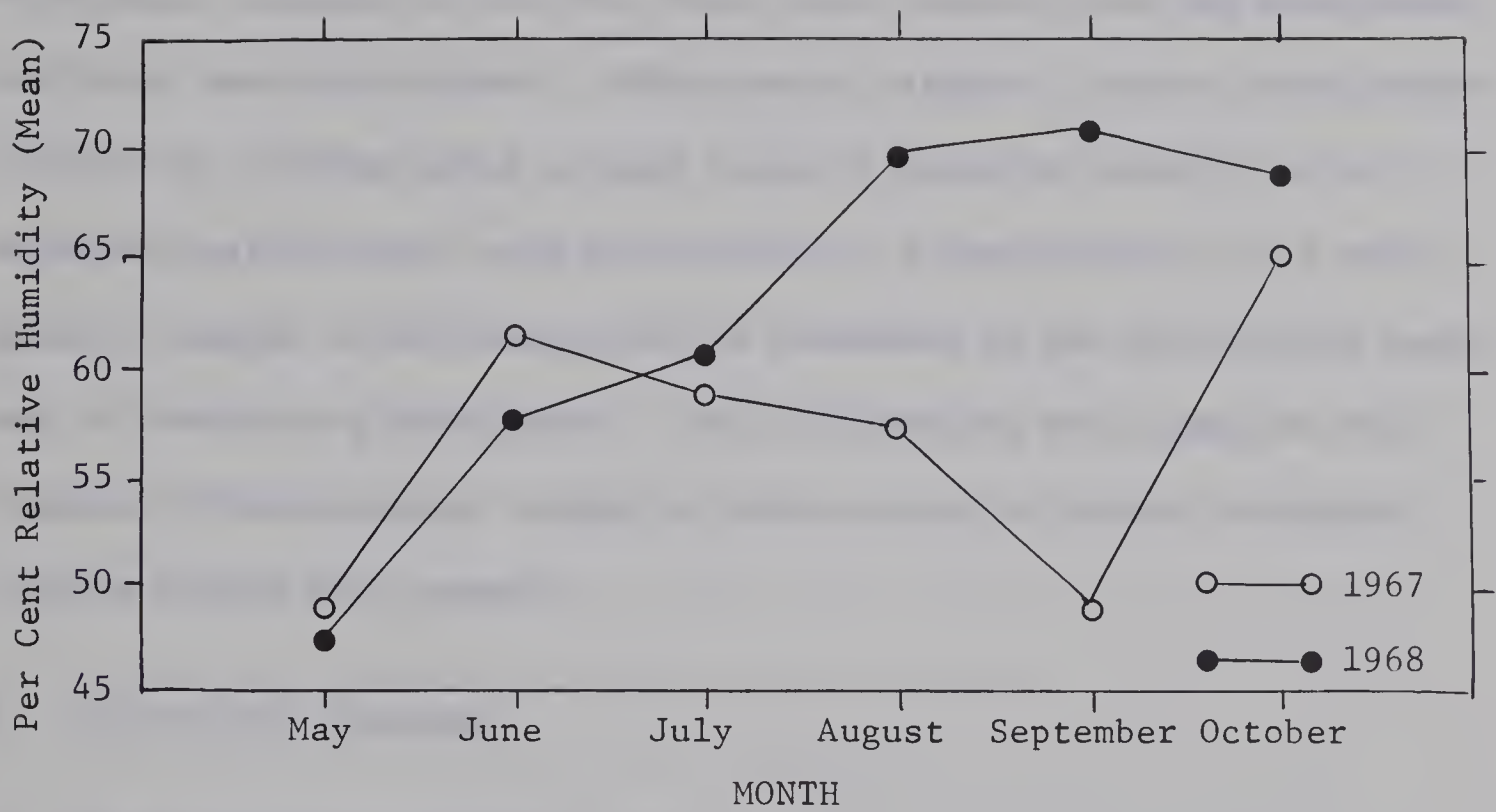


Figure 4. Average of monthly mean relative humidities recorded at 0500, 1100, 1700 and 2300 hours M.S.T. at the Edmonton Industrial Airport.





The uneven growth on the margin of basidiocarps is also evident in this illustration.

The entire surface of primordia usually exhibited renewed growth. With the formation of marginal lamellae the continued growth on the upper surface rarely occurred. Both primordia which developed in sites III and IV were observed to have this growth pattern.

At the end of September 1968, all the basidiocarps that were macroscopically examined in the four sites were removed from the substratum and their anatomy analyzed. Developmental stages of other basidiocarps in site IV, corresponding to each stage of recurrent growth in the selected basidiocarps, were also studied. A description of the ontogenetic changes in microstructure is presented in the microscopic analysis of basidiocarp development. This information was augmented with studies of developmental stages of basidiocarps collected throughout Alberta during both summers.

## B. Microscopic features

### 1. The vegetative mycelium

The physiological and morphological transformation of vegetative mycelium to produce the differentiated reproductive mycelium of the basidiocarp occurs inside the wood substratum. The establishment of a clamped dikaryotic mycelium through the union of two compatible monokaryons is a prerequisite to the development of normal fructifications. Although simple septate hyphae, possibly those of *G. saepiarium*, were found in the wood near regions of basidiocarp development, no basidiocarp or mycelium giving rise to a basidiocarp was ever observed to lack clamp



connections on its generative hyphae. The dikaryotic vegetative mycelium of *G. saepiarium* is composed of thin-walled, hyaline hyphae filled with dense granular cytoplasm. The diameters of the hyphae are extremely variable, measuring 1.5-3.4 $\mu$ . They may become quite narrow not only when passing through bordered pits and walls of tracheids and ray cells, but also in the intervals between clamp connections.

The clamp connections of the vegetative mycelium are distinctive in the genus *Gloeophyllum*. They were first observed by Falck (1909) who named them "henkelschnallen", handle clamps or medallion clamps, because of the large space between the clamp and the parent hypha, Figure 36, page 92. He correctly observed that clamps were formed by the reverse growth of a short branch which fused with the parent hypha. He was unaware of the functional significance of clamp connections which was later determined by Kniep (in Raper, 1953, p. 236). Because of the unusual morphology of medallion clamps when compared with the regular clamp connections observed by Kniep, Walek-Czernecka (1933) proposed that they originated from the dual anastomoses of bifurcate hyphal tips of two different hyphae through mutual attraction, Figure 37, page 92. Since I have observed a gradual morphological gradation of medallion clamps to normal clamps on the same hypha, this mechanism of development seems doubtful. However, I have not witnessed their actual formation, nor has the nuclear condition associated with medallion formation been determined. This situation merits further investigation.

Normal clamp connections are present on mycelium in the wood and their abundance is indicative of the transformation of vegetative hyphae to the generative hyphae of the reproductive mycelium. Vegetative hyphae with medallion clamps branch between the clamps but rarely or never form







the clamps. As the hyphae progress along or through the tracheid walls toward an opening in the wood, the frequency of medallion clamps decreases and normal clamps are formed, Figure 38, page 92. The number of hyphae per tracheid rapidly increases from two or three to many coincident with secondary branching from the normal clamp connections. The hyphae with normal clamps are more refractive, indicating wall thickening, and are usually of uniform diameter,  $1.8-2.5\mu$ . The morphological change that has taken place precedes basidiocarp formation and is evidently a physiological-morphological response to the modified environmental conditions near the surface of the wood. The mycelium, with continued growth in the more highly oxygenated atmosphere outside the substratum, undergoes further modification to provide protection from desiccation and light and, in the absence of direct mycelium-substratum contact, develops a conduction system for nutrient supply. These modifications are found in the primordium.

## 2. The primordium

Primordium is a term used here to describe the dense aggregation of hyphae on the surface of the substratum which precedes the formation of a basidiocarp. As a developmental stage between mycelial differentiation inside the wood and basidiocarp formation on the outside, the primordium is, itself, capable of producing fertile reproductive structures under certain environmental conditions. When a basidiocarp is formed, the primordium beneath it remains sterile, and is referred to as a subiculum. The manner in which primordia are formed is variable according to the path by which the generative hyphae and their differentiated branches gain access to the wood surface. The type of basidiocarp formed is



variable according to the structure and form of the different primordia.

Inside cross-sectioned logs, approximately 4 cm from the cut ends of tracheids, the generative hyphae become abundantly branched with frequent anastomoses\*. The tracheids become tightly packed with mycelium, the mycelium assuming the conformation of the tracheid, Figure 18, page 82 . Near the opening of the tracheid the generative hyphae produce great numbers of skeletal hyphae. The skeletal hyphae are consistent with the morphological definition of Corner (1953) in that they are, at maturity, unbranched, thick-walled and aseptate. They are often of great length, up to 2.5 mm, but are determinate in growth, never returning to the state of generative hyphae. Most commonly they lead the advancing growth margin by forming from the terminal cells of generative hyphae, and originate from clamp connections. Secondary branches from these clamps continue as generative hyphae to produce additional skeletal hyphae. Skeletal hyphae develop from secondary branches as well. The egress of many skeletal hyphae and their branching generative hyphae simultaneously from a number of tracheids produces a raised white, cottony mat of mycelium, the primordium. The close association of the skeletal and generative hyphae imparts a strand-like appearance to the primordium. In addition to their function in leading the growing portions of the primordium, the skeletal hyphae also provide the bulk of the primordium by deviating from their straight course to intertwine among the longitudinal strands, forming a densely compacted felt. These

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\* It is very difficult to distinguish between true hyphal anastomosis and a right-angled branching pattern which simulates anastomosis. Binding hyphae found in *Gloeophyllum saepiarium* are found to form H-shaped branches. This species has been frequently observed to form interconnected hyphae by true anastomosis in both dikaryons and monokaryons. It is most probable that dikaryon anastomosis is found in the generative hyphae in the tracheids.





skeletal hyphae are formed behind the growing margin.

The skeletal hyphae are moderately thick walled at first, flexuous, densely cytoplasmic and tapered toward the apex where the walls become thinner. They remain hyaline until exposed to light, whereupon they become yellow in color and increase in wall thickness. In the absence of light they remain hyaline, permanently so if quickly desiccated, but become brown in color when growth stops due to gradual desiccation and accumulation of staling pigments with age. If growing conditions are unfavorable, the young leading hyphal tips rupture and become twisted or coalesce to form a point. Subterminal portions with thicker walls form plasmatic septa which mark the gradual withdrawal of cytoplasm. Plasmatic septa are often evident during the maturation of skeletal hyphae. The maturation of skeletal hyphae is influenced by light and low humidity. The walls, which gradually thicken, do not collapse but the cytoplasm regresses by stages laying down as it does so secondary wall thickenings. Each stage of withdrawal is marked by a plasmatic septum that also becomes thickened and colored with age. Skeletal hyphae in several stages of development are illustrated in Figure 40, page 94.

Primordia are irregular in shape because of the variable amount of reproductive growth that issues from the tracheids. The illustrations of the developmental stages of basidiocarps from primordia in sites III and IV show this irregularity and the changes in external appearance of the leading skeletal hyphae as well. The hyphae inside the tracheids remain intact when the primordium is removed from the wood surface and have the morphological appearance of mycelial strands, 25-40 $\mu$  in diameter. The integrity of the strands is soon lost after passing into the base of





the primordium, Figure 19, page 82 .

The strands which emerge from the wood contain generative hyphae of two different diameters; those which consistently give rise to other generative hyphae and are  $2.5-3.6$  ( $4.7$ ) $\mu$ , those which produce the skeletal hyphae and in later stages, the binding hyphae, and are  $1.8-2.6$  ( $3.5$ ) $\mu$ . Clamp connections are more frequent in the strands and less frequent, at greater intervals, in strand-like associations of the primordium. The average distance between clamps of marginal hyphae on the surface of the primordium was found to be  $190\mu$ . This average was obtained by measuring four successive intervals behind terminal cells of 100 leading hyphae. Intervals between clamps at the base of the primordium averaged  $137\mu$ . The average distance between clamps in the tracheids was  $87.5\mu$ . The increase in number of clamps at the base of the primordium is indicative of secondary branching to form additional structural hyphae. The generative hyphae are at first hyaline but become yellow with age and often form thick walls. When primordia are initiated on fire-scarred timber, the fibrous nature of the strands can be seen clearly as they pass through the darkened tracheids of charred wood.

In situations where dry "seasoning" cracks extend deep into the wood, the formation of primordia on the outside is preceded by the development of dense mats of mycelium within the cracks. They progress toward the outside as finger-like plates and develop primordia, the "spaltenplatte" of Falck (1909). The mycelial plates when young are light colored and composed of skeletal and generative hyphae. With age they become slightly yellow with thickened hyphal walls and form binding hyphae. Because of their differentiated nature, the plates are easily separated from the wood surface. This condition led Falck to conclude





erroneously that they were incapable of infecting the wood, lie superficially upon it, and never penetrate below. It can be demonstrated that wood inoculated with portions of the mycelial plates becomes decayed. The wood above and below dry cracks filled with the mycelial plates is never found to lack the wood-decaying vegetative hyphae.

There is little doubt that the basidiocarps which form on the outside of the dry cracks are nurtured by means of the mycelial plates which are connected to the vast network of vegetative hyphae, as are the mycelial strands in the tracheids. The plates vary in thickness according to the size of the crack which they fill. The outer portion of the plate is indistinguishable from a primordium and is termed such when its structural hyphae undergo the modifications which are induced by light and aeration. If the plates emerge at the surface in separate segments, individual primordia and subsequent basidiocarps are formed, Figure 20, page 82. When the intervening spaces are filled with additional mycelium the primordium becomes elongate and is capable of producing elongate brackets in horizontal dry cracks or imbricate basidiocarps in vertical dry cracks, as was the case in site III. The generative and skeletal hyphae of the mycelial plates are in strand-like associations and are later interwoven with binding hyphae. The binding hyphae are of the same diameter as the generative hyphae which produce them,  $1.8-2.8\mu$ . They are thick-walled, aseptate, hyaline or light yellow and branched. The branches are variable in length,  $5-150 (300)\mu$ , and fill the interstices of the mycelium. Binding hyphae are found in the strands, plates and primordia but are infrequent in the context of the basidiocarps. They differ from Corner's (1953) definition in lacking profuse and consistently short branches, Figure 40, page 94.





Primordia are also formed on the lateral surface of the outer layer of tracheids and on the bark by a suffusion of differentiated hyphae through small mechanical breaks in the plant tissue. Injury by insects, birds and other animals also provide openings in the wood surface. The accumulation of primordial mycelium is not as abundant in these situations as when strands and plates are formed.

In my material the strand-like hyphal associations in the tracheids, mycelial plates, primordia and in the basidiocarp were not constructed in the manner described by Falck (1909). They also differ from the mycelial strands of *Merulius lacrimans* studied by Falck (1912) and Butler (1957, 1958). Although the generative hyphae become enlarged and occasionally lack clamp connections, they do not become "vascular" hyphae by the means of wall thickening, dissolution of septa and transformation of clamps to open vessels. No microscopically visible ring or spiral thickenings or moniliform trabeculae form to reinforce the walls of the swollen fungal vessels. The larger generative hyphae do not serve as a central conduction system ensheathed by intertwined, branch hyphae on all sides. This appears to be the case in mycelial strands of *M. lacrimans*, but in *G. saepiarium*, both skeletal and generative hyphae are mixed in a thread-like strand with frequent additions and separations of branch hyphae on the main axis. Multiple clamp connections which facilitate strand formation in *M. lacrimans* are never found in *G. saepiarium*. Functionally, all mycelial strands serve as nutrient conduction systems where generative hyphae are included.

The primordium is at first organized only in that it has definite strand-like associations of hyphae which grow out in all directions. They vary in size, averaging 8-25 $\mu$  in diameter. The skeletal hyphae



are quite sensitive to light and changes in relative humidity. High light intensities and low relative humidity cause them to form thick, colored walls thereby abbreviating their longitudinal growth. Since they are produced successively by the generative hyphae from below, bands of growth are seen to project beyond distinct margins of differentiated, skeletal hyphae. Differentiation usually occurs during mid-day and afternoon when low relative humidities are prevalent. With the return of higher relative humidities in the evenings and early morning, the growth from behind forms a new margin. Consistently high relative humidities and available moisture allow continuous growth for several days.

The primordium gradually thickens with secondary branching of generative hyphae to form intertwining skeletal hyphae in addition to marginal growth. The direction of the strand-like aggregations becomes gradually horizontal due to a slower rate of growth on the upper and lower surfaces. The mycelium on the upper surface is exposed to fluctuations in environmental conditions causing hyphal differentiation. The mycelium of the undersurface is more protected, a microenvironment being established, and hyphal differentiation occurs slowing marginal growth. The result is a raised primordium with a somewhat flattened uppersurface, a center portion which continues growth in a horizontal or slightly ascending direction, and a lower surface, the outward face of which is tilted forward from the base to the margin. It is on this inclined surface that the hymenophores are initiated. The development at this point is referred to as the basidiocarp.

Basidiocarp is a term used here to designate the reproductive body of differentiated mycelium that bears the hymenophores and their hymenium of sterile and reproductive elements. I have purposely excluded the





often used term "carpophore", which by its first and literal meaning refers to the stalk of a fruitbody (Snell and Dick, 1957). Even in its extended definition, this term can be reasonably applied only to the entire structure of a stipitate fructification of higher fungi. A newly formed basidiocarp with hymenophores forming in the manner described above can be seen in the center of the photograph in Figure 16, page 80. Under favorable growing conditions the transition of the primordial stage to that of the basidiocarp is rapid and no clear-cut distinction can be made between them.

### 3. The pileus and the hymenophores

Immediately preceding or concomitant with the formation of distinct hymenophores, hyphal growth in the upper portion of the basidiocarp exceeds that of the central and basal portion. The outgrowth is composed of the same strand-like associations of hyphae found in the primordium. The strands continue to be oriented in an ascendent or horizontal direction and radiate in a common plane to form a semi-circular rim, the pileus. The pileus expands by the elongation and successive branching of generative hyphae to produce additional skeletal hyphae. Growth of these elements is intermittent to form successive marginal increments, similar to those described for the primordium and illustrated in Figure 21, page 82. The upper surface of each marginal increment becomes the tomentum, the lower surface differentiates into the hymenophores, and the central portion, the context, remains as the active or potentially active region for renewed growth. The thickness of the context is dependent on the size of the primordium from which it arises. The strand-like associations in the context are not discrete. They have





numerous side branches and occasionally fuse together. They measure 8-25 $\mu$  in diameter. The margin of the context is at first loosely arranged but becomes more compact as apical growth stops and secondary growth behind the margin is continued.

The tomentum is formed by the anticlinal aggregation of generative and skeletal hyphae into numerous fascicles. These hyphae approach the surface from the context without differentiation into crusts or special layers as is the case for many other polypores. The morphological characteristics of the tomentum are found to vary according to the type of skeletal hyphae formed, the rate and duration of surface growth, and external environmental conditions. If the skeletal hyphae remain relatively thin-walled throughout their development, they form loose, flexible fascicles which impart a velvety texture to the tomentum. Fascicles containing thick-walled skeletal hyphae are more rigid. If growth-limiting conditions occur rapidly during the early stages of marginal growth, a hirsute tomentum is formed. This situation often occurs in exposed sites where growth increments are quickly terminated by low relative humidity and high insolation. A gradual termination of surface growth results in a strigose or fibrillose tomentum. This type of tomentum is similar to H. Lowag's (in Teixeira, 1962, page 70) definition of "trichoderm", a type of pileus surface whose fasciculate elements are matted into a dense tangled tissue that is no longer distinctly anticlinal in orientation. Each new growth zone of the pileus can have a surface with variable morphological characteristics because of different combinations of environmental influence and internal control on hyphal development. The erect nature of the fascicles in a hirsute tomentum as well as resupinate fascicles of a strigose tomentum can be seen





in Figures 22 and 23, page 82.

Recurrent marginal growth of the pileus was evident in the external morphology of the basidiocarp formed in site III. A longitudinal radial section of pileus 3, sectioned 30 $\mu$  thick, revealed internal zonations contiguous with those on the surface. Numerous smaller zones, not apparent on the surface are also shown, Figure 41, page 94. The macrozonations of the context correspond to the major growth increments recorded during the two year study period and are appropriately labelled in the illustration. The developmental history of the basidiocarp was reconstructed using the macrozonations as markers, and characteristics of fresh material for comparison.

Zone (a) was initially the primordium, now a subiculum. The lamellate hymenophores evident in the latter part of 1967 in zone (b) were continually lengthened throughout the summer of 1968 as successive additions of hyphae were laid down at the margin. The course of the lamellae was slightly sinuous and therefore appears to be broken and uneven in the section. The deep sulcation between macrozones was due to the initiation of growth at different levels on previously formed zones. Renewed growth failed to occur on the upper surface of macrozones but did so on the surfaces of the microzones.

Microzonation is indistinct in the older portions of the pileus. Here, the zonal pattern is obscured by the increased density of the hyphae. As was observed in fresh specimens, new growth is initiated by the intrusion of new generative hyphae from the primordium and secondary branching of established hyphae. Binding hyphae are also found in the older portions of the pileus. The mycelium often becomes so tightly packed with additions of hyphae and extracellular concretions that





further internal growth is blocked. The failure of basidiocarps to initiate new growth on the established margin of the pileus can be attributed to the failure of the nutrient conduction system to reenter the old mycelium. This also may be the reason why basidiocarps of *G. saepiarium* do not increase in size indefinitely.

The square in zone (d) represents the area photographed in Figure 24, page 84. The microzonations clearly shown here were thought to be correlated with diurnal fluctuation. Attempts to measure the small daily growth increments in other basidiocarps were unsuccessful. Marginal hyphae grew around any obstacle placed in front of them and measurements by other means were inaccurate due to contraction of the mycelium on drying during the day. Although no clear-cut connection between micro-zone formation and diurnal periodicity of growth was made through direct measurement, several morphological features did indicate the involvement of solar radiation. New hyphae formed early in the morning were hyaline. They became yellow in color when exposed to direct sunlight later in the day. The color change was slow during several successive cloudy days and the microzonations formed during this period was indistinct. Shaded portions of the basidiocarp, as in the context of resupinate basidiocarps, lacked distinct zonation. The subtle differences in pigmentation of skeletal hyphae are revealed when the hyphae are stained with fast green. In the photograph, Figure 21, page 82, greater pigmentation is seen in the hyphal tips than in the hyphae immediately following the previous growth zone. The pigmented hyphal tips indicate differentiation of skeletal hyphae and the cessation of growth in response to solar radiation and attendant high temperatures; the less pigmented portions of the zone probably formed under low light intensities and higher relative humidity.





Entire zones are occasionally observed to be dark colored as seen in Figure 24, page 84. Either marginal growth was slow and continuous during several sunny days or growth occurred during a single day continuously exposed to sunlight. The termination of growth in these zones is also marked by heavier pigmentation. The periodic initiation of growth is apparently due to internal causes that have yet to be elucidated. It was interesting to note that the number of microzones in a macrozone rarely exceeded the number of days estimated to have passed during the formation of the macrozone.

The hymenophores emerge from the middle and basal portion of the primordium or from the context of the pileus and are continuous with the expanding pileus on the undersurface. A hymenophore is defined here as the distinctive structure of the basidiocarp which bears the hymenial tissue. It is incipiently lamellate on pileate basidiocarps, although occasionally modified in later development and is polymorphic on apileate basidiocarps. The hyphal composition of the trama of the hymenophores is similar to that of the context. The diameters of the skeletal hyphae are on the average, smaller (2.2-2.5 $\mu$ ) but fit well within the range of those in the tomentum and context. No distinctive aggregation or specialization of hyphae was found above or immediately adjacent to areas of lamellar initiation. Lamellae develop in a manner characteristic of the aequihymeniiferous hymenophore described by Buller (in Singer, 1962, p. 37). They are normally positively geotropic, and despite their frequent initiation in a horizontal or oblique position, their structural units gradually descend into a vertical position. Horizontal growth from the primordium ceases and recurrent descending growth issues from the context to increase the length and depth of the lamellae. Zonation



and the strand-like associations become less distinct as hyphae become differentiated to form the subhymenium, Figure 25, page 84. The depth of lamellae varies in different basidiocarps but rarely exceeds the lower portion of the subiculum. Lamellae are of uniform thickness in early stages of growth but become narrowed at their lower margin in later development. They are initiated at a relatively uniform level in the context and evenly spaced. New lamellae are initiated 0.5-1.0 mm behind the expanding, radial margin of the pileus to maintain equal numbers per area of surface. They form as bifurcations on the ends of existing lamellae or arise as new individuals. Often additional hymenophores are formed in later stages of development as lateral branches or individual structures which occasionally fuse with existing lamellae. Various patterns of branching and fusion found near the margin of the pileus are illustrated in Figure 42, page 94. The factors responsible for the degree of branching and fusion of hymenophores to become daedaloid, poroid or irpicoid in appearance will be discussed in the section on problems in development. It is significant that the hymenophores of *G. saepiarium* are never totally poroid as in *G. odoratum*, never as thin or consistently daedaloid as in *G. trabeum*, and rarely as uniformly striate and shallow as in *G. striatum*.

#### 4. The hymenium

The slower growth rate of the strand-like hyphal associations on the lower surface of the context is the first indication of the formation of hymenial tissue. At regular intervals along the lateral and lower horizontal surface of the context, the generative hyphae stop the production of skeletal hyphae and form a subhymenial layer, three or four





hyphae deep. At the same time, the strand-like associations of the context continue to grow between these intervals gradually turning vertically downward to form the hymenophores. The subhymenial layer is continuous with the outer surface of the descending hymenophores and forms a hymenium behind the growing margin. The skeletal hyphae on the lower surface of the context just above the subhymenial layer do not descend, but grow in a horizontal direction towards the pileus margin.

The generative hyphae of the subhymenium are abundantly clamped and branch profusely to produce a compact layer of basidia and cystidia, the hymenium. Basidia are also found on the tips of elongate generative hyphae near the ends of the hymenophores, Figure 26, page 84. All basidia observed had four sterigmata. In collections of fresh basidiocarps that were kept under refrigeration, the basidia often failed to produce basidiospores but germinated to form elongate hyphal filaments, Figure 27, page 84. They formed cystidia-like elements with elongate tips when allowed to dry slowly in the light, Figure 28, page 84. Basidiocarps must be quickly dried in order to preserve the characteristics of the hymenium.

The number of cystidia in the hymenium was found to vary in different specimens and cystidia were more abundant in the older portions of the hymenium. Cystidia are at first indistinguishable from the basidia in the newly formed portions of the hymenium. They gradually elongate becoming tapered at the tip and project 15-17 $\mu$  beyond the level of the basidia. When mature, they are moderately thick-walled and hyaline or light yellow in color, Figure 43, page 94. Cystidia were frequently confused with skeletal hyphae which projected through the hymenium from the context below.



The hymenium remains as a persistent layer and periodic revival of basidiocarp growth results in the stratification of hymenial tissue, Figure 29, page 84.

### Problems in Development

Certain aspects of basidiocarp development remain problematic. For example, the reasons for changes that take place inside the wood substratum to initiate reproductive growth can only be inferred from superficial observations of changes in microstructure. This is also true of the periodic growth of the primordium and the pileus margin whose zones appear to coincide with the diurnal fluctuation of environmental conditions but may also be influenced by internal factors. The variability in morphology of the basidiocarps can be traced to differences in the primordium, the position on the substrate, and the degree of fusion of individual basidiocarps and pilei. But an explanation is still needed for the polymorphology of the hymenophores. In the environmental study no attempt was made to establish experimentally a direct correlation between any given environmental factor and the morphogenetic response of developing basidiocarps. This approach is necessarily relegated to cultural studies. However, changes in the general climate surrounding basidiocarps especially when they were extreme, were clearly reflected in particular microstructural features of the hyphae and hyphal associations. A descriptive account of these growth forms allows some hypotheses to be formulated concerning the nature of the morphological response of hyphae to environmental conditions and the overall result of these responses on the final morphology of the basidiocarp.

Apileate basidiocarps with abnormal hymenophores are formed from





primordia that are sheltered from direct sunlight. Since the heat attendant on solar radiation is also reduced, a microenvironment with higher relative humidity is created. Hymenophores that develop under these conditions arise directly from the primordium as upright, toothed projections with branched or unbranched tips. Individual segments are often fused into sinuous branching plates with lacerate edges, and appear coralloid. An example of this condition is illustrated in Figure 30, page 86, where an irregular, light-colored cluster of hymenophores formed on a log beneath a piece of bark. Although entirely covered by the bark they were not in contact with it during their development.

Two pileate basidiocarps immediately adjacent to this basidiocarp were formed in a more exposed position, with only one side shaded. Hymenophores on the shaded side are variously branched and toothed, generally projecting in a downward direction from the context. The exposed sides of the two pileate basidiocarps are illustrated in Figure 31, page 86. They are seen to the right of a third basidiocarp whose entire, upper surface was in contact with the wood. The hymenophores are lamellate in positions where no protection from the external climate was provided. The inclination of the pileus surfaces toward the light indicates the possibility that they are phototropic. Otherwise, the lack of space beneath the bark during development may account for their attitude.

A comparison of the developmental stages of basidiocarps formed in the shade with those formed in the light is instructive. The successive additions of strand-like mycelium to form primordia on the wood surface exhibit an initial photo-induced response. I have determined by experiment that the differentiation of generative hyphae to form



strands of skeletal hyphae does not occur in complete darkness. Instead long, unbranched, aseptate cells develop with thin, hyaline walls. Skeletal hyphae form on the leading margin of the primordium in the presence of light to provide a protective layer over hyphae that differentiate to become reproductive elements. The exposed hyphae of the pileus, which continues as a horizontal extension from the primordium, differentiate in response to light and alternating relative humidities to form a tomentum. The strands below continue unchanged in a horizontal direction between the tomentum and the differentiated hymenium on the lower surface of the context. The relatively uniform progression of strands toward the margin is interrupted only by a geotropic downgrowth on the lower surface of the context to form lamellae.

In shaded positions on the substratum, skeletal hyphae do not form a protective layer. The strand associations that develop are light-colored due to low light intensity. They fail to become horizontally oriented since no differentiation of a tomentum occurs in the absence of low relative humidity and high light intensity. Hymenophores that arise from the primordium do not respond to the force of gravity nor do they appear to be phototropic. No growth differential was observed in situations where hymenophores were exposed to unequal illumination. Hymenophores on the shaded sides of pileate basidiocarps are more lamellate and usually respond to gravity to some extent.

Growth characteristics of basidiocarps repositioned with their lamellae directly opposite to the force of gravity provided additional information on morphological response to environmental conditions. A sessile, dimidiate basidiocarp attached to the side of a log continued to form marginal lamellae when its position was reversed. The old





lamellae of the upper surface remained unchanged. The newly produced margin formed a tomentum on the upper surface and lamellae on the lower surface. In addition, hymenophores that were irregularly branched and coralloid in appearance descended through the hyphae of the old tomentum. It was concluded that the strand-like associations were, for the most part, normally oriented in the new margin. Their descent through the tomentum surface was irregular. Their normal orientation was disrupted by the presence of the differentiated tissue of the tomentum and uniform lamellae did not form. The final morphology of this basidiocarp is illustrated in Figure 32, page 86.

When the position of a resupinate basidiocarp was reversed no further growth occurred, Figure 33, page 86. It is believed that the arrest of growth resulted from the exposure of hyphae to adverse environmental conditions and the inability of the strand-like associations in the thin resupinate context to form a new protective tomentum and fertile surface. The failure of the ends of old lamellae to continue growth to form a new protective tomentum is due either to their differentiated nature or rapid desiccation in an exposed position.

The following conclusions are derived from observation of the varied morphological responses of the basidiocarp to changes in the external environment:

- a. The stimulus to form a hymenium occurs within the compact tissue of the primordium. It is also possible that the strand-like associations issuing from the wood are capable of forming hymenial elements. However, an aggregation of mycelium was found to precede the formation of hymenial elements in both shaded and exposed situations.
- b. The strand-like associations of the context and the hymenophores are



the elements which respond to environmental variables. The nature and orientation of these associations is also genetically controlled. The degree of branching to produce daedaloid or poroid hymenial surfaces is variable in pileate specimens with similar symmetry and position on the substratum. There were no microscopic differences in the construction of the context or the hymenophores to explain their variability under similar environmental conditions. The daedaloid appearance of hymenophores in effused-reflexed basidiocarps is due to the fusion of hymenophores descending from the pileus with those growing horizontally from the basal plate.

- c. The formation of a lamellate hymenophore is dependent on the interaction and uniform, horizontal orientation of strand-like associations in the context. Where separate basidiocarps fuse the formation of lamellae is synchronized to maintain uniform intervals of separation. The tomentum differentiates in response to environmental factors. No additional development occurs on the surface of the tomentum since environmental factors prevent further vertical growth and the main course of the strand-like associations has become horizontal. In contrast, the entire surface of the primordium revives until differentiation of hymenial tissue begins and growth is horizontally directed.
- d. When protected from above by the tomentum, hymenophores respond geotropically to form lamellae. Apileate basidiocarps form in the absence of light and low relative humidities. Hymenophores arising directly from the primordium are negatively geotropic and lack any specific arrangement. Because of the abnormal form of the pileus in partially shaded basidiocarps, strand associations lack their





usual orientation. Therefore hymenophores are irregularly formed but exhibit some geotropic response due to partial shading from above.

Figure 5. Basidiocarp on log 44 in site IV with a distinct marginal growth zone formed during the summer of 1967. (X1/3)

Figure 6. Basidiocarp on log 21 in site IV. Renewed annual growth has occurred on a portion of the old margin, the rest of the basidiocarp failing to support new growth. (X1/3)

Figure 7. Effused-reflexed basidiocarp on the end of log 20 in site IV. Note the smooth surface of the pileus. (X1/3)

Figure 8. Sessile, dimidiate basidiocarp on the end of log 44 in site IV. Note the distinct zones of the hirsute upper surface. (X1/3)



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8



- Figure 9. Basidiocarp in site III, May 20, 1968. Compare with subsequent developmental stages in Figure 10 and 11. (3/8)
- Figure 10. Basidiocarp in site III, July 27, 1968. The irregular growth of the new margin indicates the development of several imbricate pilei. (X3/8)
- Figure 11. Basidiocarp in site III, September 27, 1968. Four pilei have developed from the original elongate primordium. Margins of the periodic growth increments are conspicuously hirsute-tomentose, while the surfaces are strigose-tomentose. (X1/2)



9



10



11

Figure 12. Primordium on log 23, site IV, June 18, 1968.

Marginal growth has terminated due to differentiation of the skeletal hyphae which have become thick-walled and yellow in color. (X1/3)

Figure 13. Primordia on log 23, site IV, July 27, 1968. The previously formed primordium is growing only slightly at the margin. The new primordium is the wedge-shaped spot of white mycelium just above the old primordium. (X1/3)

Figure 14. Basidiocarp from the new primordium in Figure 13, August 29, 1968. Note that the old primordium is brown, desiccated, and apparently dead. The inner brown zone of the basidiocarp was formed August 18, 1968 and is 2.3 x 1.2 cm. Total dimensions are 3.2 x 2.1 cm. (X1/3)



12



13



14



Figure 15. Substipitate basidiocarp on log 36, site IV, August 29, 1968. Development began from a primordium June 18, 1968. (X1/4)

Figure 16. Basidiocarps on the end of log 32, site IV, August 29, 1968. Note the new, white basidiocarp in the center of the log. (X1/3)

Figure 17. Basidiocarps on the side of log 1, site IV, June 18, 1968. The new growth zone is composed of strand-like associations of generative and skeletal hyphae. (X1/3)



15



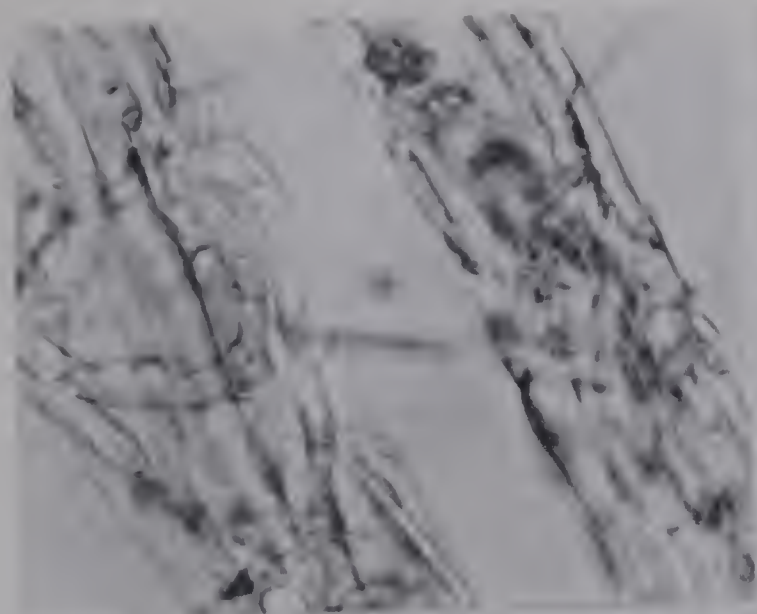
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- Figure 18. Mycelial strands from the tracheids below a basidiocarp. (X1000)
- Figure 19. Mycelial strands entering the tissue of a primordium. (X100)
- Figure 20. Basidiocarp with attached mycelial plate which formed in a horizontal dry crack of a *Populus* log. (X2)
- Figure 21. Transverse section above the hymenium showing microzones of the context composed of strand-like hyphal associations. Ends of the skeletal hyphae are deeply pigmented. (X120)
- Figure 22. Radial section of the context showing a hirsute macrozone behind several microzones. (X100)
- Figure 23. Radial section of two strigose-tomentose macrozones in the context. (X300)





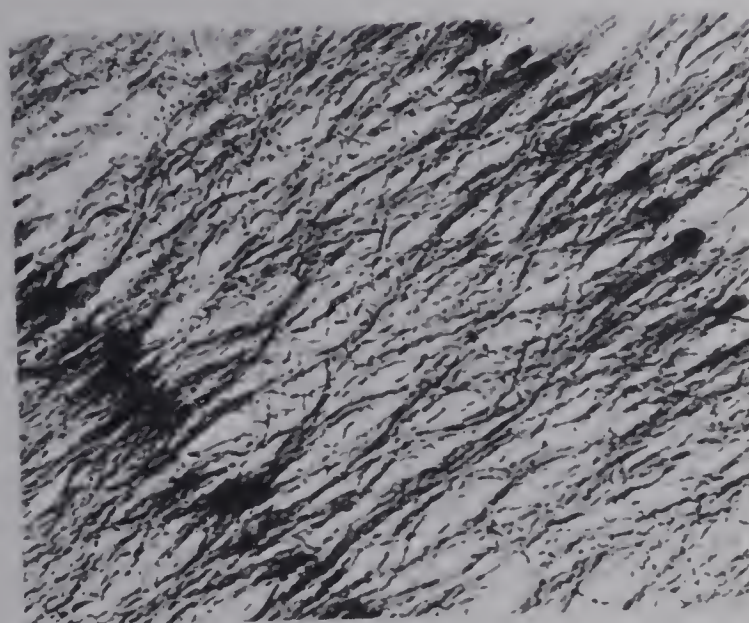
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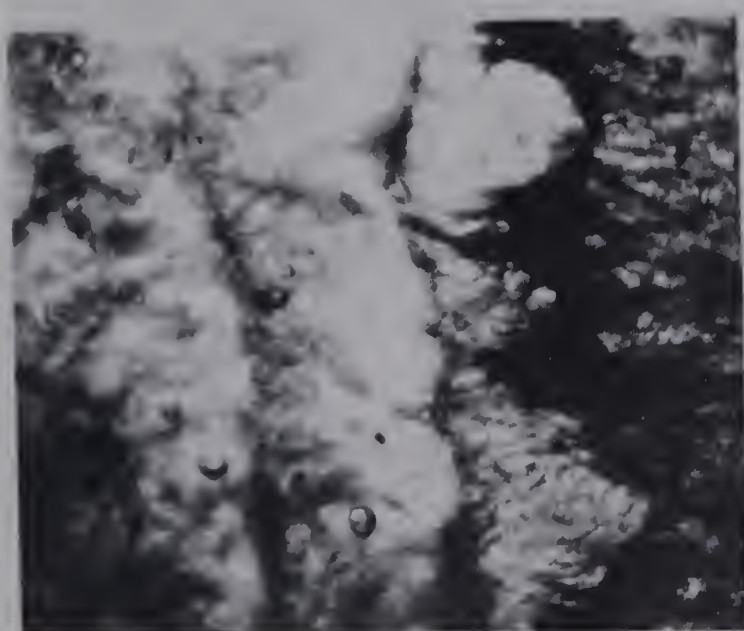
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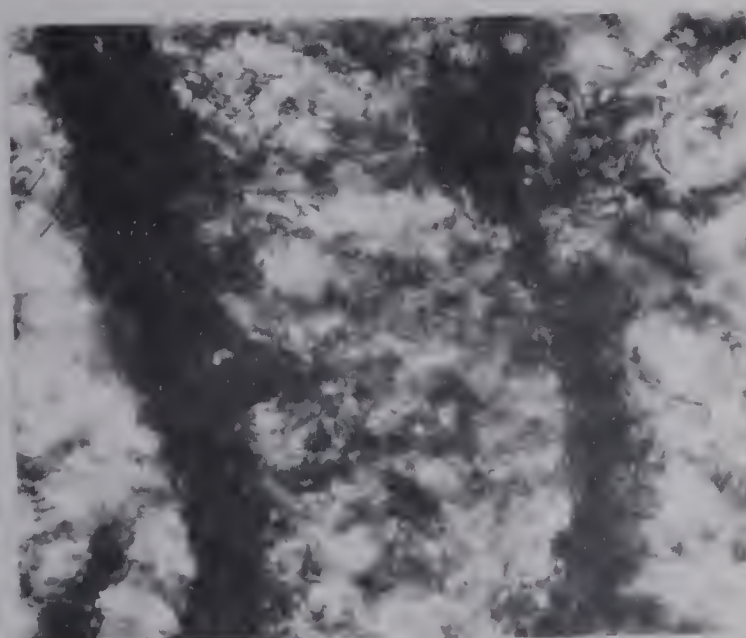
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23



Figure 24. Microzonations in the context of zone d, pileus 3 of the basidiocarp in site III. Note the pigmentation of an entire zone near the center of the section. (X35)

Figure 25. Longitudinal radial section of a pileus showing the gradual descent of the strand-like associations of the context to form a lamella. Note the more homogeneous nature of the hyphae which compose the lamellae. (X120)

Figure 26. Basidium on the end of an elongate generative hyphae on the margin of a hymenophore. (X950)

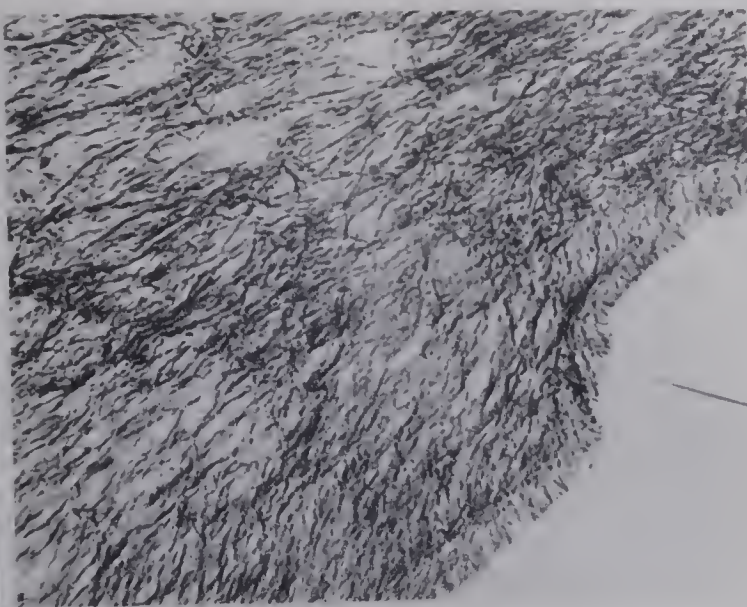
Figure 27. Germinating basidium in the hymenium of a basidiocarp that has been placed in cold storage at 4° C. (1850)

Figure 28. Cystidia-like elements with elongate tips formed from basidia. (X1000)

Figure 29. Stratified layers of the hymenium. (X800)



24



25



26



27



28



29

- Figure 30. An apileate basidiocarp and two abnormal, pileate basidiocarps that developed beneath a piece of bark. The bark has been removed (X1)
- Figure 31. The exposed surfaces of the basidiocarps seen in Figure 30. (X1)
- Figure 32. Continued growth on the pileus surface of a basidiocarp that has been rotated 180°. (X2)
- Figure 33. Resupinate basidiocarp on the undersurface of log 24, site IV. The log has been turned over. (X1/3)

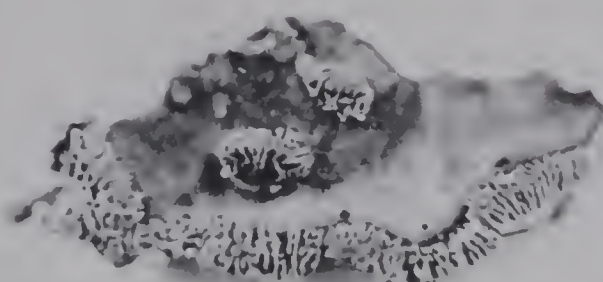




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32



33



Figure 34. Basidiocarp growth during 1967 in sites I, II  
and III. (X1)

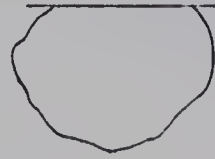
## SITE I

Increase in marginal  
dimensions in mm



June 2--12.5 x 10.0  
Aug. 1--14.0 x 10.5  
Aug. 13--21.0 x 15.0  
Aug. 25--23.5 x 16.0  
Sept. 2--25.0 x 17.5

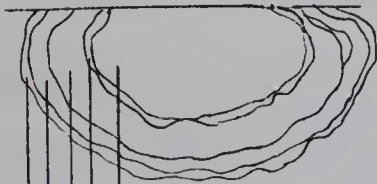
Final dimensions due  
to shrinking in mm



Oct. 8--20.5 x 15.0  
Total growth  
8.0 x 5.0

## SITE II

Increase in marginal  
dimensions in mm



June 2--24.0 x 10.0  
Aug. 1--24.5 x 10.5  
Aug. 13--30.0 x 11.0  
Aug. 25--34.0 x 15.5  
Sept. 2--35.0 x 17.0

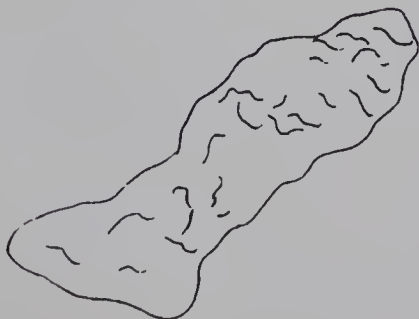
Final dimensions due  
to shrinking mm



Oct. 8--32.0 x 15.0  
Total growth  
11.5 x 7.0

## SITE III

Graduation change in dimensions with no zonation.

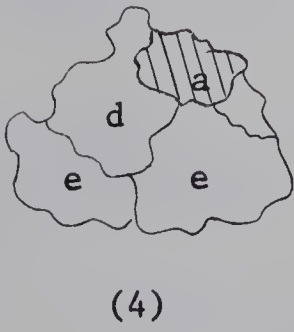
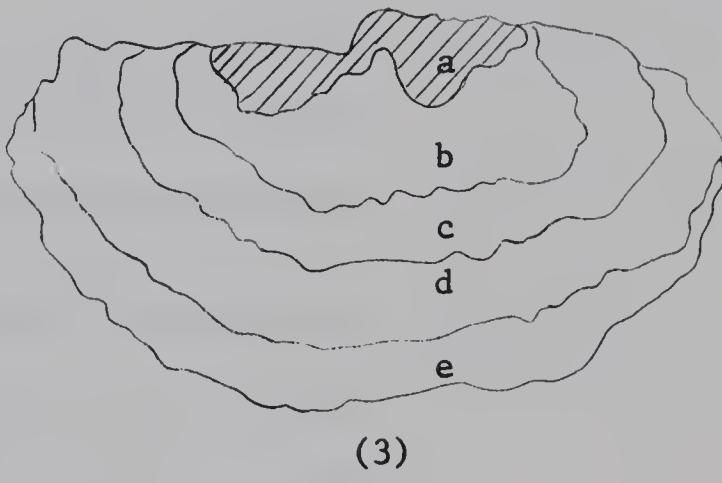
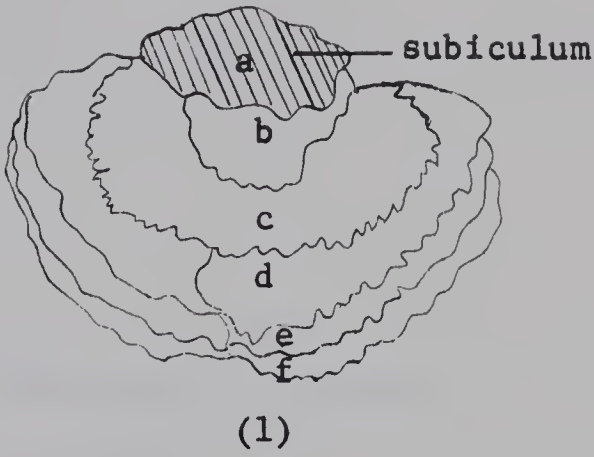
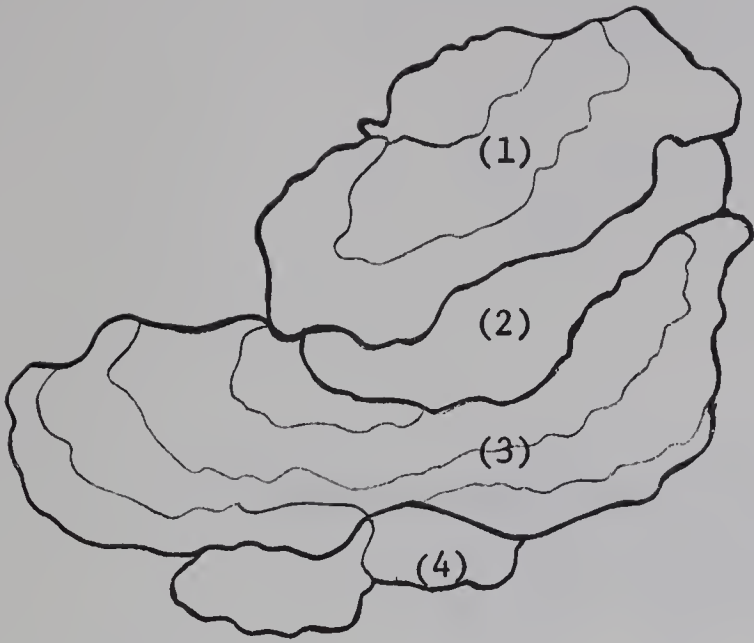


June 2--46.0 x 18.0 mm



Oct. 8--48.0 x 22.5 mm

Figure 35. Basidiocarp growth during the summer of 1968 in site III. Four pilei developed from the original primordium, now termed a subiculum. Each growth zone is lettered and the size of each zone is recorded. (X1)



Zone	Max. Diameter--mm				Date of growth
	1	2	3	4	
a	8.0	3.0	7.0	-	Present in 1967
b	5.5	7.0	9.0	-	Present in 1967
c	8.0	4.0	8.0	-	Aug. 7, 1968
d	9.0	9.5	7.0	±9	Aug. 18, 1968
e	3.0	4.8	5.0	6.0	Aug. 29, 1968
f	1.5	-	-	-	Sept. 14, 1968



Figure 36. Medallion clamps (a) and handle clamps (b).

Reproduction from R. Falck (1909).

Figure 37. Medallion clamp formation by anastomosis of

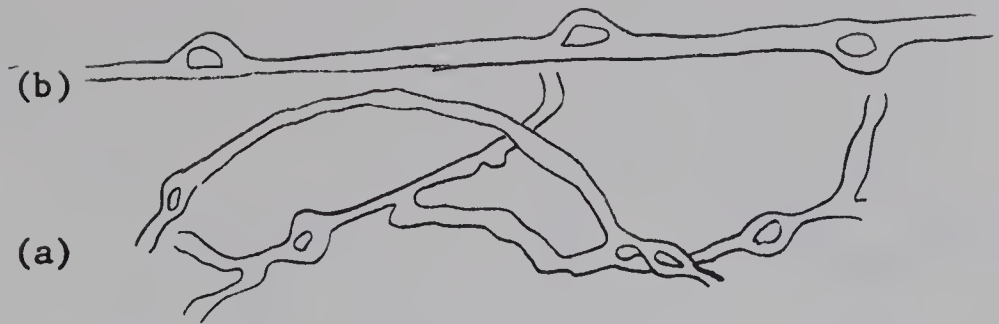
bifurcate hyphal tips. Reproduction from Walek-Czernecka (1933).

Figure 38. Gradation between medallion clamps and normal

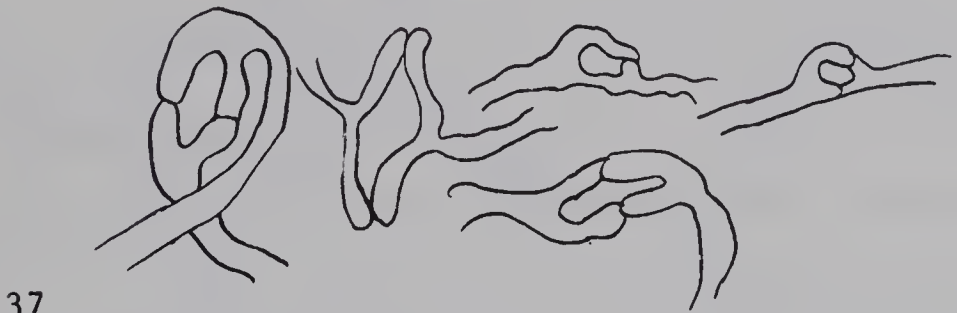
clamps on hyphae as they progress toward an opening in the substratum. (X1430)

Figure 39. Developmental sequence of a typical skeletal

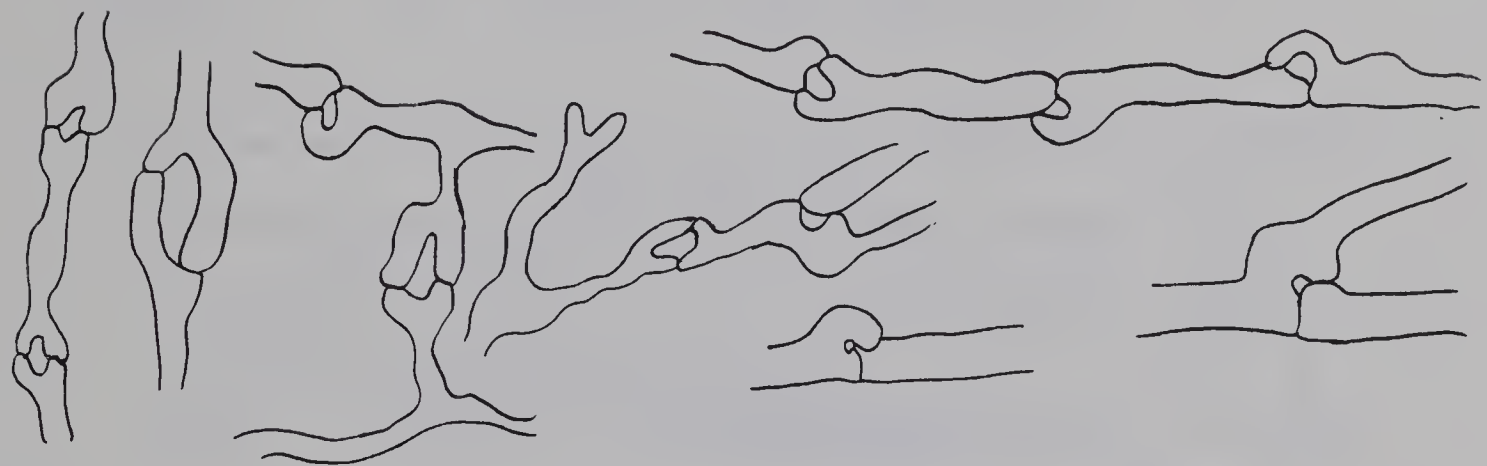
hyphae from a branched clamp connection. (X1500)



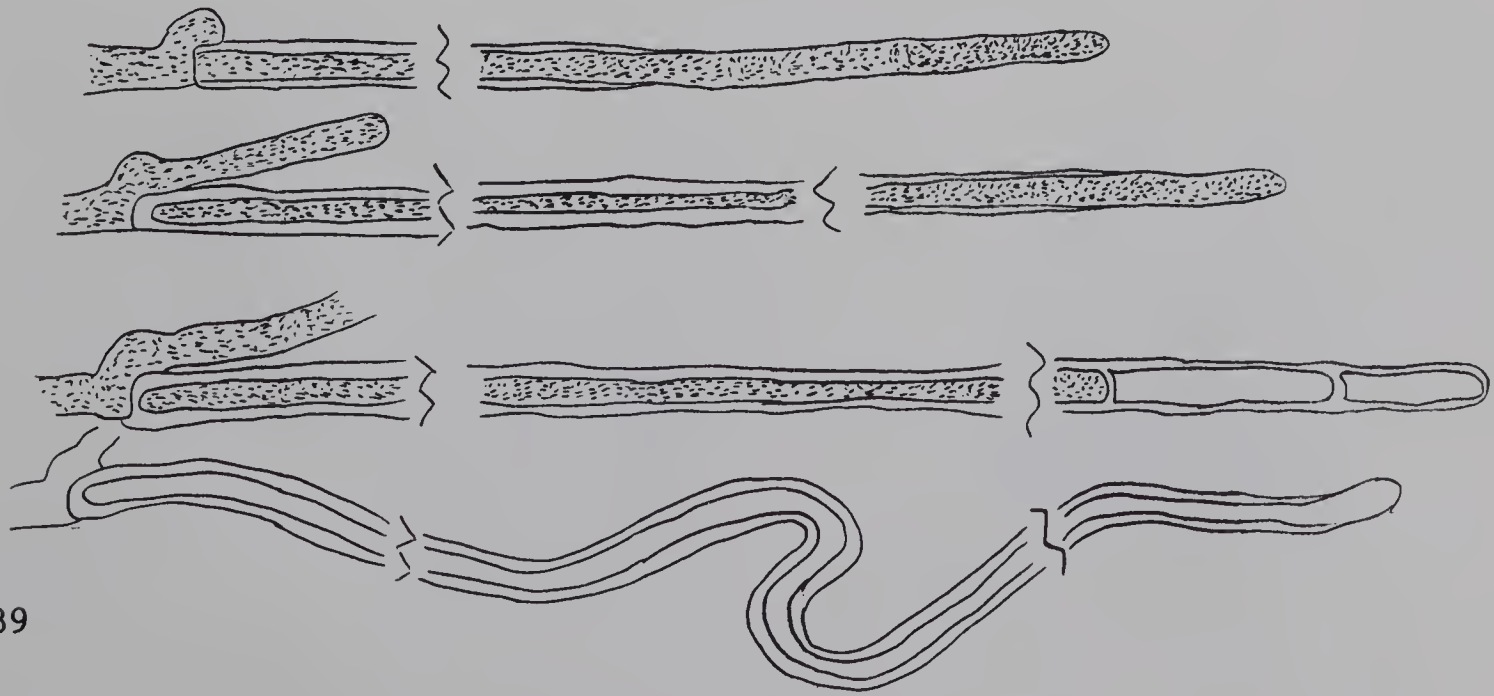
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37



38



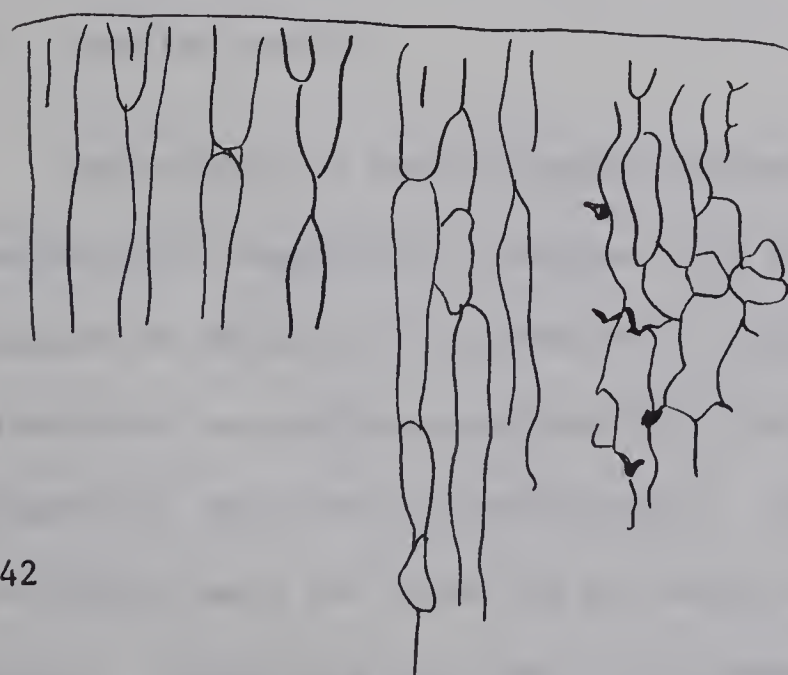
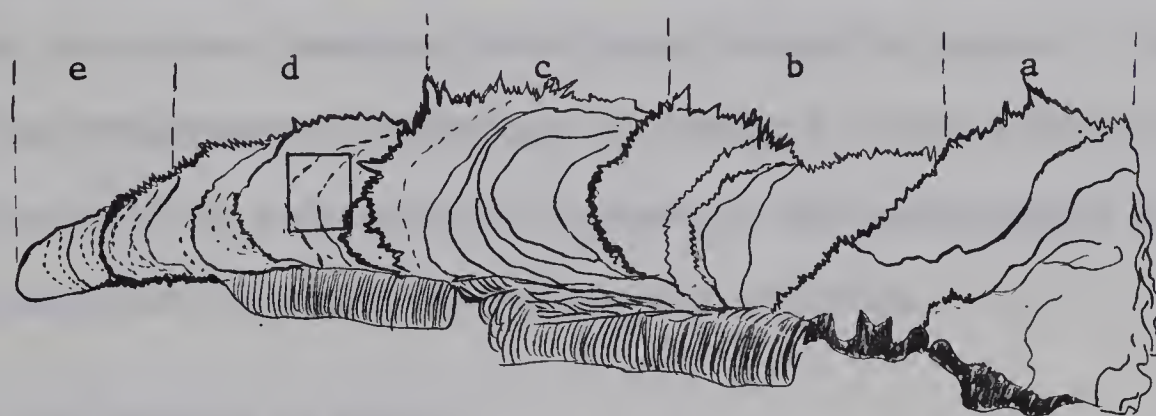
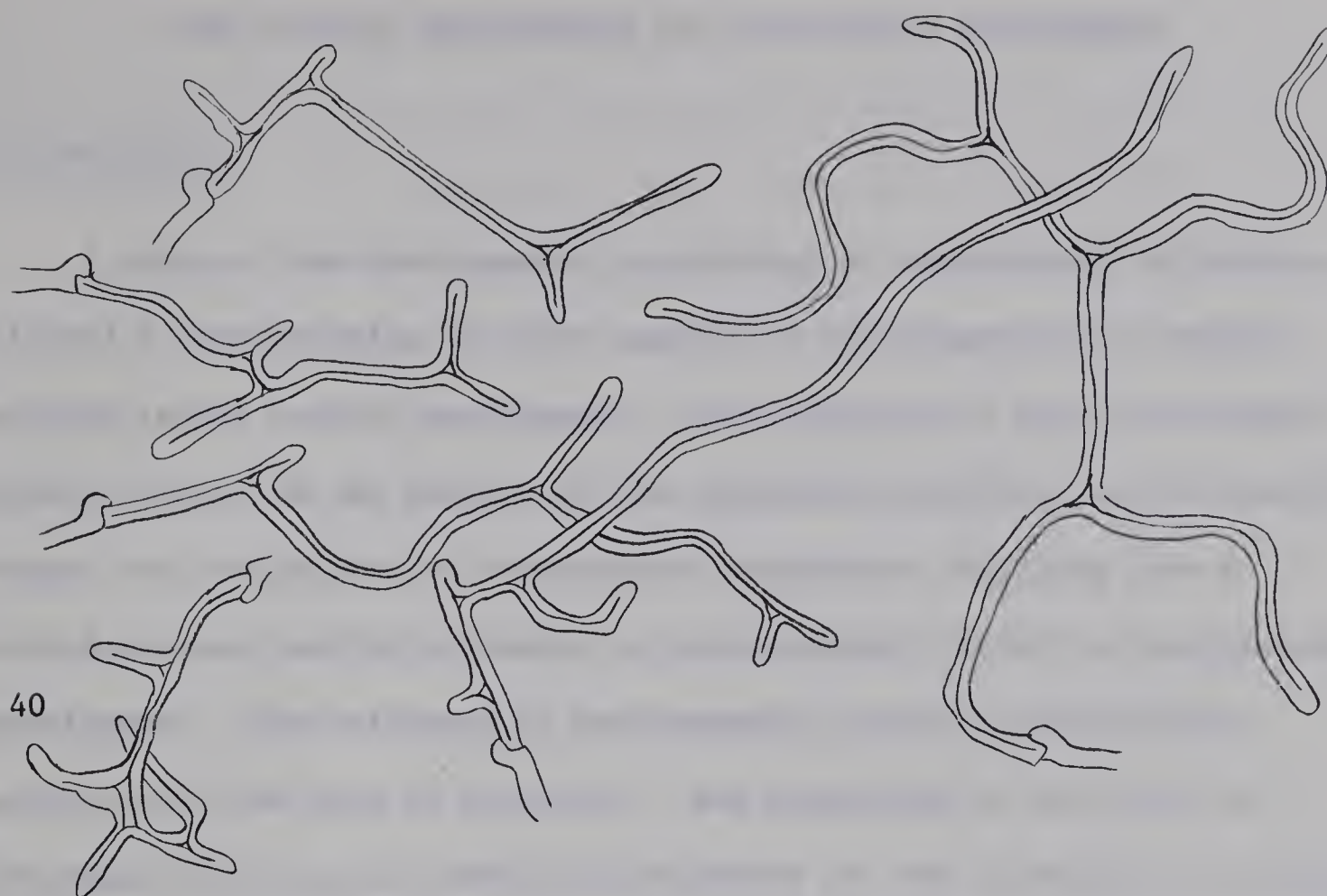
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Figure 40. Binding hyphae found in the primordium. (X1000)

Figure 41. Pileus 3 of the basidiocarp in site III.  
Longitudinal radial section. (X3)

Figure 42. Branching patterns of the hymenophores near the  
margin of the pileus. Note the initial form is  
lamellate.

Figure 43. Cystidia and basidia in the hymenium. (X1000)







## THE CULTURAL ENVIRONMENT AND BASIDIOCARP DEVELOPMENT

Introduction

A study of the developmental morphology of basidiocarps in culture allowed a consideration of three aspects of morphogenesis not easily studied in the natural environment: the influence of sexual incompatibility factors on the ability of the vegetative mycelium to form basidiocarps, the variability of reproductive structures resulting from a sexual process and the influence of environmental factors on basidiocarp development. The influence of environmental factors, particularly moisture and the type of substrate, was emphasized in an effort to determine the cultural conditions necessary for the formation of basidiocarps with structures identical with those formed in nature. A description of the developmental morphology on standard cultural conditions is presented as well as a descriptive account of the basidiocarps which developed when the cultural environment was modified.

Materials and Methods of Study

## A. Inoculum source

Inoculum from the following sources was used in the cultural study: monokaryotic vegetative mycelium from basidiospores or oidia, dikaryotic vegetative mycelium from compatible pairings of basidiospores or oidia, dikaryotic vegetative mycelium from various wood substrata and the dikaryotic mycelium of basidiocarps. The units of asexual reproduction, the oidia, were not found in any stage of basidiocarp development in nature. Suspecting that their occurrence was related to the provision of artificial conditions, I kept a detailed account of their formation in



the different cultural experiments. A list of the collections of *G. saepiarium* used in the study is presented in Appendix II together with a description of the substratum, type of inoculum and geographic location.

A system of symbols was devised to specify the type of inoculum used. Numbers represent the field collection number of the dikaryotic mycelium taken from the wood or a basidiocarp. Where a series of monokaryotic cultures was obtained from single basidiospores produced by a basidiocarp the subscript letter (s) was added to the collection number together with the number in the spore series. For example, 63<sub>s1-8</sub> represents a series of eight basidiospore cultures from basidiocarp number 63. A series of single-spore cultures from basidiocarps formed in culture was denoted by adding the letter (x) to the subscript (eg. 63<sub>sx1-8</sub>). Cultures derived from oidia produced by a cultural mycelium are labelled with the subscript (o). Since both monokaryotic and dikaryotic oidia are formed in this species, their distinction is made in the text. Matings of monokaryotic and dikaryotic mycelia are indicated by the symbols of each member of the cross.

#### B. Incompatibility factors and fruiting ability

Several series of monokaryotic cultures were obtained through the isolation of single basidiospores from basidiocarps produced in culture and in the natural environment. The spores were allowed to fall on sterile glass slides and were serially diluted using sterile, distilled water blanks. After thorough mixing, one ml aliquots of the spore suspension were evenly distributed on thinly-poured, nutrient agar plates and incubated at room temperature. Plates containing 30-60 germinated spores were selected for spore isolation. Using a Bausch





and Lomb 30 X binocular microscope with a 2X adapter and substage illumination, individual spores were located and removed with a sterile needle. Small blocks of agar containing a germinated spore were cut out and transferred to tubes of 2% malt-dextrose agar. After the mycelium had completely covered the surface of the agar slants, portions of the culture were removed and examined for the presence of clamp connections. The absence of clamp connections was taken as a reliable criterion for verifying the monokaryotic condition of individual mycelia.

Four series of single-spore cultures were prepared:  $122_{s1-10}$ ,  $60_{sx1-8}$ ,  $123_{s1-8}$ , and  $109_{s1-10}$ . Incompatibility factors were determined by pairing monokaryotic isolates of each series in all possible combinations. Pairings were made in glass petri plates containing 2% malt-dextrose agar. Small pieces of inoculum from each stock culture were placed 2 cm apart and the culture incubated in the light at 25° C for 20 days. It was found that inconclusive results were obtained if cultures were examined for clamp connections earlier than 14 days. The rate of nuclear migration appeared to be slow as indicated by the gradual formation of clamp connections. Detailed notes were made on incompatibility reaction, formation of aversion lines and the production of primordia and basidiocarps. Mycelia from the interface and the periphery of the colonies on either side of the interface were examined for the presence of clamp connections. Duplicate plates of pairings of each monospore series were made. One set was cultured for 45 days to observe the formation of fruit bodies.

Intracollection pairings of monosporous mycelium were made using isolates from widely separate geographical areas. Another series of pairings of monosporous progeny with sib-composed dikaryons and with



unrelated dikaryons was made to determine by a Buller phenomenon type reaction whether nuclear transfer, unilateral or bilateral, had any relationship to fruiting.

In the case of *G. saepiarium*, fruiting is defined as a developmental process in which the differentiation of dikaryotic and monokaryotic vegetative mycelium leads to the formation of basidia and basidiospores. The variable manifestations of fruiting in culture can be grouped into three general classes:

1. The formation of basidia on generative hyphae in the absence of differentiated skeletal and binding hyphae.
2. The differentiation of hyphae to form a raised, compact mat of mycelium, the primordium. The primordium may have fertile elements but lacks distinct hymenophores.
3. The development of a primordium to form an apileate basidiocarp with hymenophores. A fertile hymenium may be absent. The term fruit body is used synonymously with this class of fruiting.

These classes are pertinent to all descriptions of fruiting in the cultural study except in situations where normal, pileate basidiocarps were formed.

### C. Substrates and cultural methods

Malt extract agar has been used by most workers for the study of growth and reproduction of *G. saepiarium* in culture. Fructifications are regularly produced on this medium but are not typical of those found in nature. A series of experiments was conducted to observe and describe the effect of different substrata on fruiting. Three general classes of





media were used. (1) Liquid media containing either basal synthetic additives or 2% malt extract. (2) Solid 2% agar containing one of the following nutrient sources: 2% malt extract, carboxy-methyl cellulose, Whatman's cellulose powder and filter paper cellulose. The cellulose media were supplemented with basal synthetic additives. (3) Natural wood substrates from both hardwood and softwood sources in the following variations: different grades of sawdust with or without Badcock's preparation (1941), blocks of wood which were sterilized and pre-infected wood blocks cut from logs containing viable vegetative mycelium of *G. saepiarium*. A complete list of formulae for media used in the cultural study is included in Appendix III.

The order of the experiments beginning with liquid media, passing through agar-based preparations and ending with the natural wood substratum was intentional. After reviewing a number of papers dealing with culture of wood-decay fungi, I concluded that a wholly or predominantly liquid substratum (such as agar) was inherently artificial. A continuous supply of water is available to the fungus in agar. Water is limited and intermittent in a natural wood substratum. There is also an artificially produced atmosphere in standard culture vessels. Relative humidity and gaseous composition of the air are modified and there is an accumulation of staling products both in the air and in the substratum. Many fungi, particularly lower fungi, can reproduce normally under a variety of circumstances, but the morphogenetic phases of the higher fungi come into expression under different, and often contrasting, sets of stimuli which are apparently quite precise. It is reasonable to provide environmental conditions which most closely approach those in nature. Environmental factors considered in the study were: temperature, relative humidity,





moisture of the substratum, light, and accumulation of staling products.

## 1. Liquid media

The vegetative growth of *G. saepiarium* on a liquid, basal synthetic medium has been reported by HacsKaylo et al. (1954), but the formation of basidiocarps has not been described. An experiment was designed to compare the differences in the amount of growth and formation of fruit bodies by different mycelial inocula cultured on either a liquid basal synthetic medium or a liquid malt extract medium. The inoculum was obtained from pure cultures of 63, 116, 114<sub>s7</sub>, and 114<sub>s8</sub>. Uniform disks of mycelium, 3 mm in diameter, were removed from the margin of each culture and transferred to matched 250 ml Erlenmeyer flasks containing 50 ml of cold-sterilized medium. The flasks were cleaned in a dichromate-acid solution and rinsed several times in tapwater, then in distilled water and oven-dried at 350° F for 12 hours. They were closed with sponge plugs covered with a strip of Parafilm (American Can Company) to prevent excess evaporation during the culture period.

The pH levels of two groups of each medium were modified to determine the influence of pH on pigmentation and fruiting. Monospore isolates frequently were found to produce a diffusible brown pigment that appeared to be associated with a staling reaction. Isolate 114<sub>s8</sub> was selected for study because of its ability to produce the pigment while 114<sub>s7</sub> does not. Pigmentation is common in dikaryotic cultures particularly when fruiting is initiated. To prevent an anticipated drop of four pH units from neutrality, determined by preliminary growth experiments, maleic acid was added to one batch of each medium to provide a buffer capacity of 0.05 moles/50 ml. These media were adjusted to a





pH of 6.4 with KOH as was another batch of media without the buffer. The pH of a third batch was left unaltered.

The cultures were incubated in a growth chamber at 25° C and exposed to a 12 hour light and dark cycle with a light intensity of approximately 160 foot-candles. All quantitative data on growth in the various media are based on the average dry weight of mycelium produced in three replicate flasks after 20 days of growth. Dry weights were determined using the following procedure. Separate disks of Whatman's filter paper (No. 3) were washed, dried and weighed. The mycelium from each flask was collected separately on a filter paper disk placed in a millipore filter apparatus by drawing the medium through the filter paper with a vacuum. The filtrate was washed with distilled water, dried in a hot air oven at 90° C to a constant weight and placed in a desiccator jar. The weights of the filter paper and the mycelium were recorded using an analytical balance. Qualitative descriptions of fruiting are composites of the replicate cultures and are ranked according to the classification listed on page 99. Changes in pH of the media were recorded with a Beckman pH meter.

An additional experiment using liquid 2% malt extract medium was carried out to evaluate the influence of aeration, relative humidity and light on the formation of fruit bodies. An experimental apparatus similar to that used by Plunkett (1956) was set up. Mycelia from cultures of 63 and 114<sub>s7</sub> were transferred to 4 oz glass jars filled with medium. The jars were placed in a large desiccator vessel. Duplicate cultures were prepared. A continuous source of medium was supplied from a central reservoir outside the vessel and a constant supply of sterile humidified air was passed through the culture vessel at a rate of 120 ml



per minute. The air was humidified by bubbling it through saturated salt solutions and water to provide humidities of approximately 70%, 85% and 98%. Separate experiments were carried out for each relative humidity. Cultures were grown at a temperature of 25° C for 6 weeks with an alternating 12 hour light and dark cycle at intensities of 40 and 160 foot-candles.

## 2. Agar media

*G. saepiarium* is reported to decay the cellulose portion of the natural wood substratum in preference to the lignin fraction but there have been no reports of basidiocarp formation on a synthetic medium using cellulose as a carbon source. Several cellulose sources were substituted for glucose in standard basal synthetic medium in agar. Ten grams of either carboxy-methyl cellulose (CMC), Whatman's cellulose powder (WCP), or filter paper cellulose (FPC) were added to separate media. In a batch of medium containing CMC two grams of yeast extract were added instead of thiamine and biotin (CMCY). Portions of the aerial mycelium from cultures of 50, 63, 113, 60<sub>sx1</sub> and 114<sub>s8</sub> were transferred to 10 cm petri plates containing the various media. Cultures were also plated on malt extract agar for comparative purposes. Duplicate plates were made for all cultures. The cultures were incubated in a growth chamber providing 8 hour days at 25° C and 16 hour nights at 18° C for one month.

In a second group of experiments the surface of malt extract agar, contained in either petri plates or petri jars, was covered with a disk of differentially permeable cellophane. Since the mycelium was not capable of penetrating the cellophane, it was possible to study growth





and fruit body formation by a vegetative mycelium removed from the essentially liquid environment of the agar. Small vials of 12% KOH were supplied to remove CO<sub>2</sub>, a possible staling factor, from the atmosphere of the culture vessels. Mycelium from cultures of 63, 113 and 114<sub>s8</sub> and germinated basidiospores of 63<sub>sx</sub> were used as inoculum. Four replicate cultures were made from each inoculum source. Two were placed in the dark and two in the light, cycled as in the previous experiment. One culture of each pair was supplied with KOH. A duplicate sequence of cultures without cellophane disks inoculated with mycelium from 63 and 114<sub>s1</sub> was used as a control. Cultures were incubated at 25° C and examined after 14 days. Those cultures grown in the dark were removed and allowed to continue growth under laboratory conditions for an additional 14 days. Laboratory conditions include the diurnal fluctuation of diffuse light from windows near the cultures and the ambient temperature and relative humidity of the room.

### 3. Natural wood media

#### a. Plunkett apparatus and wood blocks

Sterilized wood blocks from poplar, spruce, fir and pine were saturated with water, placed in an upright position in eight ounce jars half filled with wet absorbent cotton and autoclaved. They were inoculated with mycelium from isolate 63. The jars were incubated at 25° C and supplied with a continuously renewed air supply using the apparatus of Plunkett (1956). The relative humidity of the air was maintained at approximately 85% by bubbling air through a KCl solution. Sterile distilled water was periodically added during a two month growth period to maintain a constant supply of moisture in the wood. Light, cycled to give 8 hour days and 16 hour nights with intensities of 160 and 40





foot-candles in two separate experiments, was provided using one jar of each culture per experiment.

b. Lohwag method

A batch of sawdust-accelerator medium was packed in sterile canning jars and inoculated with portions of mycelium from cultures of 50, 113 or 114<sub>s7</sub>. The jars were closed with a Bakelite lid, that was loosely secured to allow gas exchange and the egress of mycelium. This cultural procedure was similar to that used by Lohwag (1955). Duplicate cultures were prepared and incubated for two weeks in the dark at 25° C. At the end of this period, one set was placed in a growth chamber and grown for six weeks at a temperature of 25° C in continuous light (intensity 160 foot-candles) and a relative humidity of 85%. The other set was cultured under laboratory conditions.

c. Tamblyn-DaCosta method

Badcock's accelerator medium was added to 100 grams of sawdust from either poplar or pine wood. The cultural procedure of Tamblyn and DaCosta (1958) was followed. Twelve eight ounce glass jars two inches in diameter with a one and one-half inch neck were tightly packed with the medium so as to barely exceed the rim of the mouth. A slightly larger jar was inverted to cover the opening of the culture jars and the entire apparatus was autoclaved. Vegetative mycelium from cultures of 63<sub>sx</sub>, 113, 50 and 122<sub>s9</sub> was placed on the surface of the sawdust in a jar. Three replicate jars were prepared for each inoculum source and the cultures were incubated in the dark at room temperature. After the sawdust became covered with vegetative hyphae in four to six weeks, a sterilized block of either pine or poplar wood, previously saturated in water, was placed over the neck of the culture jar in place of the glass cover. Parafilm





(American Can Company) was used to seal the exposed surface of the wood to prevent excess evaporation and the junction of the wood and the glass neck was sealed with paraffin. When the mycelium became visible on the outside of the wood block, the protective covering was removed and the blocks and jars were placed in an open tank. Absorbent cotton was placed in the bottom of the tank around the bases of the jars. The cotton was saturated with water until the moisture level came within two inches of the necks of the jars. A deep well was cut in the top of the wood and kept continuously moist by the addition of distilled water.

d. Pre-infected wood pieces

(1) Collection 119

Twenty small wood blocks, 3" x 1/2" x 1/4", were cut from a white spruce log heavily infected with *G. saepiarium*. The blocks were washed several times in sterilized distilled water, surface sterilized by immersion in 70% ethyl alcohol and flamed until the surface of the wood became slightly charred. Each block was placed on the surface of a thin layer of malt extract agar contained in petri jars or plates. Fifteen of the block cultures were placed on a desk top in the laboratory and allowed to receive diffuse light from a nearby window. The other five cultures were placed in cardboard boxes sealed with tape to exclude light.

(2) Collection 100

A large block of pre-infected wood, was placed in a tray and provided with several different combinations of atmospheric humidity and moisture in the substratum while cultured under the light and temperature conditions of the laboratory.

(3) Collection 122

Five, one foot sections of spruce logs, six inches in diameter, were



arranged in various positions in a fume hood. They were heavily infected with *G. saepiarium* and had numerous basidiocarps on the bark. The basidiocarps were removed and the logs were soaked with distilled water. The logs were incubated at room temperature with a light from an overhead lamp providing a maximum intensity of 80 foot-candles. The relative humidity was periodically raised with water applied as a fine mist from above by forcing a jet of water through the small aperture of an aspirator flask. Several combinations of external environmental factors were applied according to the response of the mycelium during basidiocarp development.

The procedures followed using collections 122 and 100 were based on the results of previous experiments and upon the response of the mycelium during the culture period. For this reason, separate accounts of methods and results of experiments using these collections are presented following a discussion of results from the genetic and cultural experiments listed above.

### Results and Analysis of Incompatibility Factors and Fruiting Ability

The results of pairing monokaryotic isolates 122<sub>s1-10</sub> in all possible combinations are arranged to give a bipolar mating pattern in Figure 44a, page 108. The evidence of bipolarity is based on the presence or absence of clamp connections. Clamp formation was noticeably irregular in several of the pairings: few normal clamps were found, but pseudoclamps and simple septa on mycelia at the colony interfaces were abundant. On the basis of clamp formation regardless of type, a perfect bipolar pattern of interfertility was exhibited. A tetrapolar table distinguishing four incompatibility types was constructed when pairings





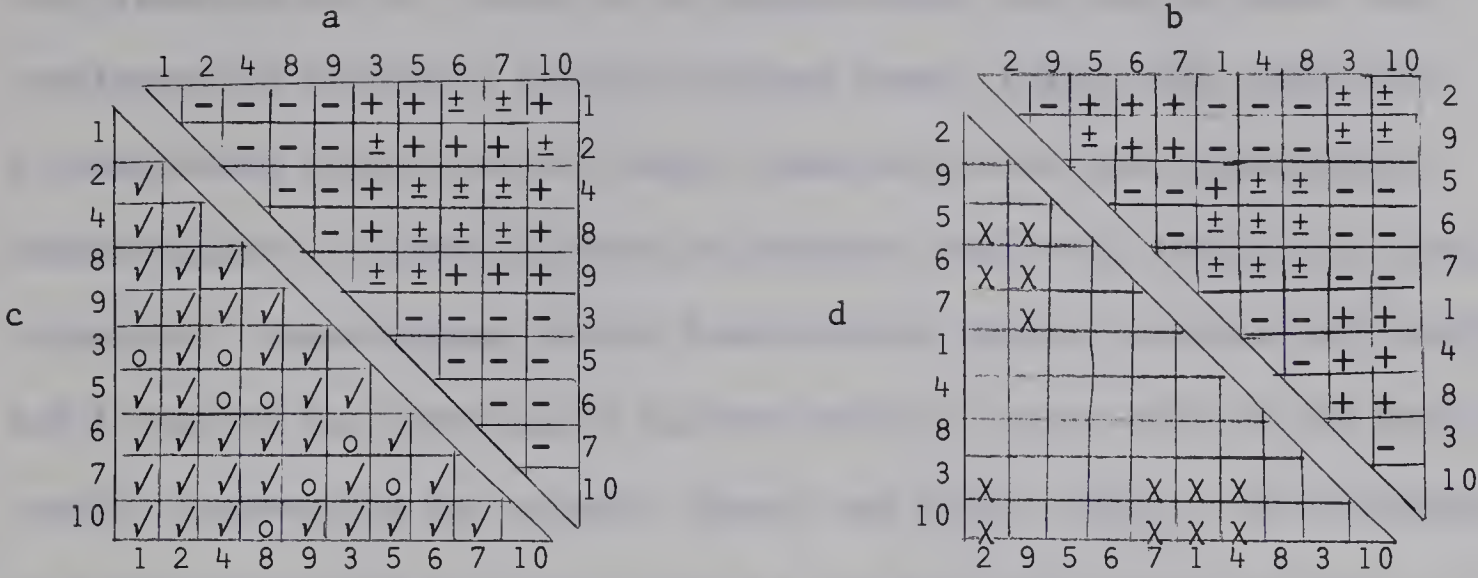


Figure 44. Tables showing reactions of paired cultures of 122<sub>s1-10</sub>.

- a. Arrangement of mating reactions in a bipolar table based on presence or absence of clamp connections. Clamp reaction: + predominantly clamp connections, ± predominantly pseudoclamps and simple septa with occasional clamps, - entirely simple septate.
- b. Arrangement of mating reactions shown in Table a in a tetrapolar table based on clamp characteristics.
- c. Table showing presence of aversion lines. ✓, aversion line formation; ○, no aversion line formation.
- d. Table showing pairings of Table b that formed primordia or fruit bodies. X, fruit bodies or primordia.



that resulted in the formation of pseudoclamps and simple septa were considered to represent distinct mating types, Figure 44b, page 109. A conspicuous feature of A-B factor interaction is the requirement of heterozygosity for both factors to initiate hook cell fusion and clamp formation. Pseudoclamps result from crosses between mycelia with different A factors but identical B factors and are formed only at the zone of contact between the two mycelia (Raper and Raper, 1968). The formation of pseudoclamps was not restricted to the zone of contact in most of the pairings of 122<sub>s</sub>. There also appeared to be a difference in the degree of irregular clamp formation exhibited by each mate. In the pairings of 5 with 8, pseudoclamps were found throughout the mycelium of 5, while 8 remained simple septate except at the interface. Unilateral clamp formation was noted in other pairs as well.

Differences were noted in the intensity of the aversion lines formed by the interface hyphae of paired cultures. Mycelial aversion was indicated by a distinct line of demarcation between the paired mycelia, and by pigmentation of the hyphae and substratum at the zone of contact. Aversion lines have been found to be associated with A-B factor interaction in tetrapolar fungi. Brodie (1936) showed that an aversion line developed between incompatible or compatible monokaryon or dikaryon mycelium of *Lenzites betulina* when matings differed in the B factor. The reaction was referred to as a "barrage". Papazian (1950) showed that the barrage reaction in *Schizophyllum commune* was due to the establishment of a common-B heterokaryon and was not developed in any other combination. An attempt to correlate the formation of aversion lines with mating factors in the 122<sub>s</sub> pairings was unsuccessful. They formed irrespective of mating type when considered either as tetrapolar





or bipolar, and were common in incompatible pairings, Figure 44c, page 108.

Formation of fruit body primordia closely paralleled the establishment of normal clamp connections, Figure 44d, page 108. Fruit body primordia were recognized by the formation of a dense, raised mat of intertwined skeletal and generative hyphae. Two pairings formed sterile primordia on mycelium that had numerous pseudoclamps but possessed many normal clamps as well. Several paired cultures with numerous pseudoclamps formed raised tufts which were similar in gross morphology to fruit body primordia. However, the tufts were comparatively soft and lacked the internal differentiation of generative hyphae to form thick-walled skeletal hyphae and basidia. Three pairings which exhibited normal clamp development failed to fruit.

Mating reactions of eight monokaryotic mycelia of 60<sub>sx</sub> are arranged to form a bipolar pattern of incompatibility in Figure 45, page 111. Pairings of 3 with 6, 7 and 8 resulted in few clamp connections and numerous pseudoclamps and simple septa. Clamp connections were more frequent in pairings of 4 with 6, 7 and 8 but pseudoclamps were common throughout the cultures. These reactions are opposed to the formation of true clamp connections in pairings of 3 and 4 with 1, 2 and 5. Simple septa and pseudoclamps were found only on marginal mycelia of these pairings. Formation of aversion lines between pairs of monokaryons was variable and not related to mating type. Examination of pairings after 45 days of growth revealed that in no case were fertile fruit bodies produced. Primordia were few and reduced in size or absent. Pairings differed from one another in growth rate, amount of surface mycelium and pigmentation. The pigment was brown in color, diffusible and associated with staling. Staling was recognizable by marked degeneration



	1	2	5	6	7	8	3	4
1		-	-	-	-	-	+	+
2	-		-	-	-	-	+	+
5	-	-		-	-	-	+	+
6	-	-	-		-	-	±	+
7	-	-	-	-		-	±	+
8	-	-	-	-	-		±	+
3	+	+	+	±	±	±		-
4	+	+	+	+	+	+	-	

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	1	3	5	8	2	4	6	7
1		-	-	-	+	+	+	+
3	-		-	-	+	+	+	+
5	-	-		-	+	+	+	+
8	-	-	-		+	+	+	+
2	+	+	+	+		-	-	-
4	+	+	+	+	-		-	-
6	+	+	+	+	-	-		-
7	+	+	+	+	-	-	-	

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	10	1	3	4	6	7	8
2	±	±	±	±	±	±	+
9	+	±	±	±	±	±	±
5	±	±	+	+	+	+	±

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Figure 45. Mating reactions of paired cultures of 60<sub>sx1-8</sub> arranged in a bipolar table.

Clamp reactions: + predominantly clamp connections, ± predominantly pseudoclamps and simple septa with occasional clamps, - entirely simple septate.

Figure 46. Mating reactions of paired cultures of 123<sub>sx1-8</sub> arranged in a bipolar table.

Color in the lower half of the table denotes fruit body formation in one or both members of each compatible mating. Symbols for clamp reactions are similar to those of Figure 45.

Figure 47. Compatible mating reactions of paired cultures of 109<sub>s1-10</sub>.

Color denotes fruit body formation. Note the unilateral formation of normal clamps in compatible pairs. Symbols for clamp reactions are similar to those of Figure 45.





of hyphae and clamps and reduction of aerial growth in areas of heavy pigmentation.

Primordia and fruit bodies were formed in all but two compatible pairings of eight monokaryotic isolates of 123<sub>s</sub>, Figure 46, page 111. Although mycelia of the pairs were clamped, the formation of fruit bodies was often unilateral. Isolates 2, 7 and 8 consistently formed fruit bodies while 1, 3 and 5 formed fruit bodies only when mated with 8. The presence of aversion lines was conspicuous in many of the pairings, but again was not related to incompatibility factors.

Unilateral fruiting was associated with unilateral clamp formation in paired cultures of 109<sub>s1-10</sub>, Figure 47, page 111. Fruiting occurred only in compatible pairings involving 2 and 5 in situations where normal clamps were formed.

Dikaryotization of monokaryons by sib-related dikaryons did not occur. Monokaryons paired with unrelated dikaryons were not fully dikaryotized as indicated by the formation of pseudoclamps even when pairings were made of isolates from widely separate geographical areas, Table V, page 113. Dikaryons frequently intermingled with the monokaryon making it difficult to determine the precise mating reaction at the interface. It is possible that dikaryotization is more frequent than reported here. However, it was noted that haploid sectors developed in dikaryotic cultures and remained so, indicating that the Buller phenomenon proceeds very slowly or not at all. A similar situation was reported in *Lenzites trabea* by Barnett and Lilly (1947) and in *Fomes cajanderi* by Adams and Roth (1967). In di-mon pairings, no monokaryons in which dikaryotization was thought to occur, were found to produce primordia or fruit bodies.

Inter-collection pairings of 122<sub>s1-5</sub> and 60<sub>sx1-3</sub> with 46<sub>s1</sub>, 50<sub>s1</sub>,



Table V. Di-mon pairings of monosporous progeny with sib-composed and unrelated dikaryons. Clamp reactions are recorded for each mate after one month of associated growth.

Pairings		Clamp Reaction	
	122 <sub>s</sub>	Monokaryon	Dikaryon
4 x (4+6)		-*	±
4 x (4+10)		-	+
5 x (4+6)		-	±
5 x (4+10)		-	+
2 x (4+6)		-	±
2 x (4+10)		-	+
3 x (4+6)		-	±
3 x (4+10)		-	+
<hr/>			
60 <sub>sx</sub>	122 <sub>s</sub>		
3 x (2+3)		-	±
3 x (1+10)		±	+
3 x (2+5)		-	+
7 x (2+3)		-	±
7 x (2+5)		-	+
7 x (1+10)		±	±
<hr/>			
114 <sub>s</sub>	122 <sub>s</sub>		
1 x (4+10)		-	+
3 x (4+10)		-	+
7 x (4+10)		-	+

\* Clamp reaction: + predominantly true clamps, ± predominantly pseudoclamps and simple septa, - entirely simple septate





63<sub>sxl</sub>, 109<sub>sl</sub>, 114<sub>sl</sub> and 116<sub>sl</sub> resulted in complete compatibility. Clamp formation was unilateral in cases where staling prevented clamp development.

Later in the study four monokaryotic isolates were found to produce fertile basidia on raised mats similar in morphology to the mycelial mats formed in colonies with simple septa and pseudoclamps. The basidia and spores were of normal shape and size but were rarely produced. Attempts to isolate single basidiospores were unsuccessful. The occurrence of basidia on monokaryotic mycelia was not surprising since monokaryotic isolates of *G. trabeum* often produce haploid fruit bodies in culture. Leonard and Dick (1968) demonstrated the induction of haploid fruit bodies in *Schizophyllum commune* by the application of a chemical substance isolated from dikaryotic fruit bodies of the same fungus. A similar attempt was made in the present study using extracts from both *G. trabeum* and *G. saepiarium*. Fruiting occurred in 63<sub>sxl</sub> but not in any other isolate tested. This isolate was found to produce basidia in the absence of the extract as well.

The results of the genetic experiments indicate that fruiting ability in *G. saepiarium* is not necessarily linked to the establishment of the dikaryotic condition in the mycelium. The manifestation of the fruiting response is related to the dikaryotic condition as indicated by the abundance and type of clamp connections formed and the amount of differentiated hyphae to form fruit bodies. The more conspicuous, fertile fruit bodies were formed on mycelium that was predominantly clamped. Variability in fruiting of dikaryons may be attributed to cytoplasmic factors and/or heteroallelic conditions of the mating factors. The striking unilateral formation of clamps and fruit bodies, as



well as the ability of certain isolates to fruit more consistently in all pairings lend support to this contention. The possibility of somatic recombination as a cause for the morphological heterogeneity of paired cultures is not ruled out. Parag (1968) has directly observed the somatic fusion and division of nuclei in *S. commune*. This phenomenon has yet to be shown in heterothallic bipolar fungi.

The nature and the function of sexual incompatibility factors in *G. saepiarium* require further study. Tetrapolar mating patterns have been mistaken for bipolar patterns due to the failure to recognize the characteristic mating reaction of common-B heterokaryons in the contact zone as pointed out by Aschan (1952) and Macrae (1967). However, pseudo-clamps are not restricted to this zone in pairings of *G. saepiarium*. Barrage formation, usually associated with incompatibility factors in tetrapolar fungi as well as bipolar fungi (Adams and Roth, 1967), did not bear the same relationship in this fungus.

### Results and Analysis of the Cultural Experiments

#### 1. Liquid media

The results of the cultural characteristics of different inocula on various media are summarized in Table VI, page 116. Fruiting occurred in dikaryotic cultures of 116 and 63 on both basal synthetic (BS) and malt extract (ME) media. The monokaryotic cultures of 114<sub>s7</sub> and 114<sub>s8</sub> did not fruit and produced less mycelium on both media. Fruiting in cultures of 116 was poor on ME and somewhat better on BS, while 63 fruited well on both media and formed comparatively larger fruit bodies and primordia. Since, in all cases, fructifications produced were similar to the abnormal growth forms found on malt agar, their structures will





Table VI. Cultural characteristics of mycelium after 20 days of growth on liquid malt extract and basal synthetic media with modified pH levels.

Inoculum Source	Medium and Modification*		Dry Wt.** mg	Pigment	pH**	Fruiting Class ***
116	BASAL	1	5.86	yellow	3.80	2
	SYNTHETIC	2	6.32	none	3.70	3
		3	6.81	yellow	4.20	2
	MALT	4	11.23	none	3.55	none
	EXTRACT	5	13.65	none	3.20	1
		6	19.22	none	3.40	1
63	BASAL	1	12.41	yellow	3.85	2
	SYNTHETIC	2	10.25	brown	4.10	2
		3	14.33	yellow	4.25	3
	MALT	4	28.80	none	3.30	3
	EXTRACT	5	34.84	none	3.80	3
		6	41.62	none	3.85	3
114 <sub>s7</sub>	BASAL	1	8.42	none	3.96	none
	SYNTHETIC	2	7.96	none	4.00	none
		3	9.60	none	4.40	none
	MALT	4	10.78	none	3.51	none
	EXTRACT	5	10.43	none	4.12	none
		6	12.20	none	3.64	none
114 <sub>s8</sub>	BASAL	1	3.63	brown	4.85	none
	SYNTHETIC	2	3.45	brown	5.10	none
		3	5.58	brown	4.50	none
	MALT	4	10.14	brown	3.48	none
	EXTRACT	5	10.96	brown	3.71	none
		6	11.36	brown	3.65	none

\* Basal synthetic medium 1. no buffer, initial pH 4.04  
 2. no buffer, adjusted pH 6.4  
 3. buffer, adjusted pH 6.4  
 Malt extract medium 4. no buffer, initial pH 4.75  
 5. no buffer, adjusted pH 6.2  
 6. buffer, adjusted pH 6.2

\*\* Mycelial dry weights and pH values are averages of three replicate flasks.

\*\*\* Fruiting class: 1. with basidia only  
 2. with primordia  
 3. with apileate basidiocarps



be described together. It was interesting to note that the mycelium of the larger fruit bodies of class 3 was heavily saturated with water and, as the fruit bodies increased in size, many sank below the liquid surface, the mat being incapable of supporting their weight. Viable basidiospores were produced on all class 3 fructifications.

All cultures on ME had faster growth rates as indicated by comparing dry weights of the mycelium. Otherwise, differences in growth appeared to be due to individual differences of the inocula. The lesser amount of growth by 116 when compared to 63 may be related in some way to the adaptations of each to the substratum from which they were originally obtained. Isolate 116 was collected from poplar wood and 63 was collected from pine wood. Diffusible pigment in the agar did not seem to be associated with a corresponding decrease in growth. The buffer failed to maintain the pH at the adjusted level. Its capacity was either exhausted or the fungus utilized it as a metabolite. The mycelial weights were generally higher on all media with adjusted and buffered pH indicating the possibility that growth was more rapid at the higher pH levels provided earlier. No consistent differences in pH were found in cultures with a diffusible pigment in the medium. Heavily staled cultures grown for two months still retained a pH level between 3.0 and 4.5.

Fruit bodies formed on ME under different light intensities and relative humidities provided by Plunkett's apparatus were similar in morphology to those found in the preceding experiment. Light had no apparent effect on the structure of fruit bodies at the intensities provided. In cultures provided with a 70% relative humidity, the pigmentation of the primordia was considerably darker when exposed to light intensity of 160 foot-candles than when exposed to 40 foot-candles.





Pigmentation was reduced in all cultures at high relative humidities. It was concluded that pigmentation is enhanced by light under dry conditions. Primordia that developed under a 70% relative humidity were not raised but consisted of a dense tough mat of heavily pigmented skeletal and binding hyphae. The surface hyphae were desiccated and no fertile elements were formed. No fructifications were observed on mats of monokaryotic mycelia, while oidia were abundant. In marked contrast, primordia and fruit bodies of 63 grown under 80% and 98% relative humidities were lightly pigmented, raised and possessed fertile elements. Oidia were also abundant, particularly in cultures in the nearly saturated atmosphere. The fruit bodies of all cultures were saturated with moisture which could be easily expressed. Contamination was a constant problem using this cultural method.

## 2. Agar media

The cultural characteristics of various inocula grown separately on several cellulose sources added to basal synthetic medium are summarized in Table VII, page 119. All isolates grew poorly on CMC, FPC and WCP and no fruiting occurred. The colony diameters were greater on WCP due to the extensive ramification of widely separate hyphae but little aerial mycelium was produced. Oidia were common in all cultures and variable in abundance. Submerged hyphae were noticeably degenerate, often fragmenting or lacking cytoplasm in intercalary segments, and simple septa and pseudoclamps were common in dikaryotic cultures. The diffusible, brown pigment of 114<sub>sg</sub> was not produced on these media. With the addition of yeast extract to CMC, more mycelium was produced in all cultures, especially at the point of inoculation, but no fruiting





Table VII. Cultural characteristics of mycelium on basal synthetic agar with different cellulose sources and malt extract agar.

Inoculum Source	Type of Medium*	Colony Diam.** cm	Pigment	Oidia	Fruiting Class***	Other Cultural Characteristics
50	CMC	5.2	none	++	none	sparse mycelium, fragmented; clamps normal
	CMCY	9.0	none	++	none	moderate mycelium, some pseudoclamps
	FPC	3.8	none	+++	none	sparse mycelium, vacuolate; clamps degenerate
	WCP	8.5	none	++	none	thick mycelium at center; clamps degenerate
	ME	9.0	none	+	2	dense mycelium, clamps normal
63	CMC	4.0	none	+	none	sparse mycelium, vacuolate, fragmented; clamps rare
	CMCY	8.0	none	+++	none	moderate mycelium; good clamps
	FPC	6.5	none	++	none	sparse mycelium, fragmented; pseudoclamps
	WCP	9.0	none	++	none	dense mycelium at center; fragmented at margin
	ME	9.0	yellow	+++	3	dense mycelium; some simple septa at margin
113	CMC	5.5	none	+	none	sparse mycelium, vacuolate; clamps and pseudoclamps
	CMCY	8.2	none	++	none	mycelium at center, some fragmentation
	FPC	6.0	none	+++	none	moderate mycelium; clamps and pseudoclamps
	WCP	8.5	none	++	none	moderate mycelium; pseudoclamps
	ME	9.0	yellow	+++	3	dense mycelium, brown hyphae; good clamps
114 <sub>s8</sub>	CMC	2.8	none	+++	none	sparse mycelium, mixed, fragmented or intact
	CMCY	8.4	none	++	none	submerged mycelium, moderate covering of oidia
	FPC	3.6	none	++	none	very sparse mycelium, degenerate; clamps rare
	WCP	8.0	brown	+	none	sparse mycelium, vacuolate, fragmented
	ME	8.2	brown	+	none	mycelium and oidia dense at center
63 <sub>sx1</sub>	CMC	6.0	none	+	none	little visible growth, oidia at center
	CMCY	8.5	none	++	none	moderate mycelium, submerged, covering of oidia
	FPC	2.0	none	++	none	little visible growth, oidia at center
	WCP	9.0	none	+	none	sparse mycelium, vacuolated, fragmented
	ME	8.5	slight	+++	none	patches of dense oidia and mycelium
* CMC carboxy-methyl cellulose						** Data are averages of duplicate plates examined after 30 days growth.
CMCY carboxy-methyl cellulose and yeast						
FPC filter paper cellulose						
WCP Whatman's cellulose powder						
ME malt extract						
*** Fruiting classes: 2 - primordia						
3 - apileate basidiocarps						





occurred. Pigment was formed in the cultures of 114<sub>s8</sub>. In contrast, the growth on ME agar was luxuriant and many primordia and fruit bodies were formed. In a later experiment using an agar basal synthetic medium with glucose, primordia and fruit bodies developed. It is probable that *G. saepiarium* lacks the necessary enzymes to successfully utilize cellulose in the form provided under cultural conditions.

The characteristics of mycelium from different sources grown on ME agar in varied environments are summarized in Table VIII, page 121. Fruit bodies were not formed in the dark. Pigmentation was common in older, staled cultures and was seen both in the walls and in the lumen of the hyphae. Few recognizable skeletal hyphae were found, although some moderately thick-walled, aseptate unbranched, hyphae of great length were present. Growth rate, as indicated by colony diameters was faster in the dark. Cultures grown in the dark and then placed in the light were found to produce fruit bodies and primordia. The addition of KOH to absorb CO<sub>2</sub> had no apparent effect on pigment production or fruit body formation. Colony diameters were smaller in all cultures containing KOH and/or cellophane disks indicating that both these factors retarded the growth rate. No differences in the structure of primordia and fruit bodies were noted when the vegetative body of the fungus was removed from direct contact with the agar medium.

Differences in the ability of replicate plates to produce fruit bodies from primordia as well as colony diameters were observed. In an additional experiment it was shown that inoculum taken from oidial portions of stock cultures usually had a faster growth rate in terms of colony diameter, but required a longer period to initiate a fruiting response. Inoculum including portions of differentiated mycelium from



Table VIII. Cultural characteristics of mycelium grown on malt extract agar for 14 days at 25° C in varied environments.

Inoculum Source	Cellophane Disc	Culture Vessel	KOH	Light	Colony diam. cm	Fruiting Class**	Pigment in Agar	Oidia*
113	+	jar	+	+	3.4	2	yellow	+++
	+	jar	+	-	5.2	none	none	+
	+	jar	-	+	4.0	2	none	+++
	+	jar	-	-	4.8	none	brown	+++
	+	plate	+	+	3.8	2	brown	++
	+	plate	+	-	4.5	none	none	++
63	+	jar	+	+	3.4	2	brown	+
	+	jar	+	-	5.0	none	none	+
	+	jar	-	+	4.9	3	brown	+
	+	jar	-	-	5.4	none	none	++
	-	jar	+	+	6.8	2	yellow	++
	-	jar	+	-	6.5	none	brown	+++
	-	jar	-	+	7.0	3	none	++
	-	jar	-	-	6.6	none	yellow	+++
114 <sub>s8</sub>	-	jar	+	+	5.8	none	brown	++
	-	jar	+	-	6.2	none	brown	++
	+	jar	-	+	4.0	none	brown	++
	-	jar	-	-	6.3	none	brown	++
63 <sub>sx1</sub>	+	plate	-	+	2.0	none	none	++
	-	plate	-	+	4.8	2	none	++
	-	plate	-	-	5.2	none	none	+++

\* Abundance of oidia qualitatively evaluated by observations.

+ few oidia, ++ moderate oidia, +++ abundant oidia

\*\* Fruiting classes: 2, with primordia only; 3, with basidiocarps





primordia or fruit bodies present in the stock cultures fruited faster and had slower growth rates. It was concluded that inoculum from reproductive mycelium is predisposed to fruiting while inoculum from vegetative mycelium requires a longer period for fruiting to occur.

The sequence of morphological changes in the structure and arrangement of the mycelium from a vegetative state to a reproductive state was similar in both liquid and agar media. The formation of basidiocarps was most easily observed on agar media and is described here as a composite of many observations of cultures grown under the several cultural conditions provided.

The hyphae that emerge from the dikaryotic mycelium and from the germination of dikaryotic oidia inoculated on the various media are at first simple septate. Clamp formation occurs behind the advancing margin. No medallion clamps were found in mycelium in liquid or agar cultures. Clamp connections are infrequent on mycelium submerged in liquid and agar media and are rarely formed beneath glass slides placed over the hyphae on the margin of the colony. The hyphae were found to bear clamps within several days after the slide was removed. Dikaryotic mycelium responded in a similar fashion to anaerobic conditions provided in an experiment by David (1968).

The development of hymenial elements was not restricted to stages of fruit body development. Dikaryotic mycelium growing from ME agar onto a non-nutrient agar surface formed basidia, cystidia and oidia in a linear arrangement along the same hypha. Clamp connections were often degenerate or absent at the base of these elements. Many of the basidia were fertile and are illustrated in Figure 48, page 135. The cystidia were thick-walled, very irregular in shape, occasionally branched at the





apices, and covered with a non-wettable substance, possibly calcium, since it dissolved in KOH. A typical cystidium is illustrated in Figure 49, page 135. It was often difficult to distinguish between these elements when they were in intermediate stages of development.

The hyphae of the vegetative mycelium are at first very sparse and produce abundant oidia in six to ten days. The shape and size of the oidia often vary with each isolate. They are formed as fragmented portions of the vegetative mycelium or as single terminal segments on oidiophores. The oidia become increasingly abundant with the age of the culture. Behind the advancing margin clusters of thickly branched hyphae are formed. Large coils of hyphae also develop that branch in all directions as illustrated in Figure 50, page 135. The coils and hyphal aggregates are found in both dikaryotic and monokaryotic mycelia and are the only distinct features in early stages of hyphal development that indicate a type of specialization that could possibly be associated with fruit body formation. No direct connection was made between them and fruiting because their presence was obscured by the dense mat of mycelium formed prior to the initiation of primordia. As hyphae accumulated to form a mat by extensive secondary branching, certain areas become elevated to form tufts or mounds, the primordia. The primordia are usually hemispherical and vary greatly in size due to time of initiation and growth rate. Occasionally they cover large portions of the mycelial mat. They are at first snow white in color but later become yellow to amber brown. The coloration is enhanced by irradiation under low relative humidities and is variable in individual primordia. They are composed of loosely intertwined skeletal, binding and generative hyphae and the difference between skeletal and binding hyphae in culture





is not as apparent as it is in nature. Since generative hyphae frequently branch, their distinction is based on the large cell diameter and darkly pigmented walls. The average hyphal diameters of each hyphal system are similar to those found in nature: generative hyphae,  $1.4-4.4\mu$ ; binding hyphae,  $1.7-2.8\mu$ ; skeletal hyphae,  $2.6-3.8\mu$ . Cell walls were frequently thicker in culture, often occluding the lumen. Because of the extensive branching of the hyphae in the primordium, the strand-like associations of generative and skeletal hyphae are infrequent and are not oriented in any particular fashion. This aspect is illustrated in Figure 51, page 135. The surface hyphae of the primordium was often fasciculate when exposed to low relative humidities as illustrated in Figure 52, page 135. However, the fascicles did not form a pileus as did the fascicles in nature. There was little indication of internal zonation so clearly evident in primordia in the natural environment.

In three or four weeks hymenophores emerged from the primordia. They had the appearance described by several authors as "lettuce-like" as illustrated in Figure 53, page 137, or stalked with forked horn-like projections as illustrated in Figure 54, page 137. In contrast to the primordium, hymenophores were more colored and were composed of tightly compact generative and skeletal hyphae. A longitudinal cross section of various hymenophores showed that no strand-like associations were present, Figure 55, page 137. Transverse sections compared to longitudinal sections demonstrated a definite orientation of hyphae in the direction of growth, Figure 56, page 137. In some instances there was a definite phototropic orientation of hymenophores when provided with unilateral illumination, Figure 54, page 137, but this response was not consistent in many of the cultures. Hymenophores are often more typically lamellate in cultures which were inverted upon inoculation. This positional response did not occur in cultures inverted after





primordia were initiated. It is likely that the mycelium which is responsible for the initiation of hymenophores was horizontally oriented due to the physical barrier of the substratum and the development of the hymenophores was therefore lamellate. Serial sections of the mat failed to provide conclusive evidence for this hypothesis, but artificial conditions of culture could have provided an additional modifying influence on hyphal orientation.

Basidia developed over the entire surface of the upright hymenophores. Their size was quite variable,  $22.4-110.7 \times 6.4-10.2\mu$ , and often they were unevenly arranged in the hymenium. Some basidia with only two sterigmata were noted. No cystidia were found, although occasional thin-walled, septate, sterile elements were interspersed among the basidia. Basidiospores were of normal size and shape.

Monokaryotic fruit bodies were found only on agar media. The primordia were composed of loosely arranged hyphae and the mat was easily dissected. Differentiated hyphae with thick, colored walls were noticeably lacking. No distinction could be made between skeletal and binding hyphae since there was abundant branching of all differentiated elements and diameters were very variable. No distinct hymenophores were observed. The basidia formed from branching generative hyphae at the surface of the primordium in the manner illustrated in Figure 57, page 137. They were variable in length,  $25-120\mu$ , were rarely fertile and bore four basidiospores of normal dimensions.

The mycelia of 63, 63<sub>sx1</sub> and 122<sub>s9</sub> were stained with HCl-Giemsa to determine the nuclear condition. The nuclei were clearly stained only in the portions of each colony which had formed oidia. It was interesting to note that numbers of nuclei per oidium varied from one to over thirty





in all three isolates. The nuclear condition of 122<sub>sg</sub> is illustrated in Figure 58, page 137. Where nuclei were seen in the intact vegetative mycelium, a similar multinucleate condition was observed. The maximum diameter of over 150 discrete nuclei of each isolate was measured. The average diameter of nuclei of monokaryotic isolates was averaged 1.73 $\mu$ , slightly larger than that of dikaryotic isolate 63, 1.42 $\mu$ . The multinucleate condition is particularly interesting in view of the fact that a heterokaryotic condition could easily be established in the oidia. Oidial colonies differ markedly in colony characteristics and ability to fruit. It may well be that the heterogeneity of neohaplonts found in tetrapolar fungi (Aschan, 1952) is also related to a multinucleate condition. Griffin and Wilson (1967) discovered that the nuclei in conidia of *Fomes annosus* varied from one to ten and vegetative cells contained as many as 30 nuclei.

### 3. Natural wood media

#### a. Plunkett apparatus and wood blocks

Fruit bodies were evident on the block cultures in Plunkett's apparatus after three weeks of growth. Only dikaryotic cultures formed fruit bodies and primordia, although all isolates thoroughly decayed the wood medium. Medallion clamps were found in the tracheids. The surface of the wood was entirely covered with white mycelium which became pigmented in areas where primordia developed. Fruit bodies were formed equally well on poplar wood and wood of conifers cultured under low and high light intensities. They were stalked and possessed a microstructure similar to primordia found on agar and liquid media. Oidia were present but not in great numbers.



#### b. Lohwag method

Preincubation in the dark, provision of an enriched natural medium, exposure to continuous light, and a high relative humidity did not induce the formation of normal basidiocarps. Most of the cultures became contaminated in the constant high humidity provided by the growth cabinet. Mounds of mycelium were formed in the space between the lid and neck of the jar but no fruit bodies were produced. Even less growth occurred under laboratory conditions although contamination was not a problem and moisture was available in the medium. The most plausible explanation for the lack of growth was the dry atmosphere of the laboratory.

#### c. Tamblyn-DaCosta method

The results using this method were encouraging. Raised primordia appeared on the lower surface of the wood blocks inoculated with dikaryotic cultures in three to four weeks in all cultures that were free from contamination. A basidiocarp developed from one of the primordia at the edge of a wood block near the neck of the glass jar. Thin sections of the wood revealed abundant mycelium with medallion clamps in the tracheids. The hyphae were desiccated near the openings of the tracheids on all parts of the wood surface except that part near the moist well. A typical pileus was not formed, but hymenophores were covered above with irregular groups of differentiated hyphae. Similar abnormal basidiocarps were observed in nature. The structural elements of the primordia and hymenophores were comparable to those formed in nature and there was some indication of internal microzonation in the mycelium. No oidia were formed. The significance of these results will be discussed later.





#### d. Pre-infected wood pieces

##### (1) Collection 119

In the light, established dikaryotic mycelium in wood blocks first emerged from the cut ends of the tracheids and from the wood in contact with the agar. A few blocks were contaminated with other fungi, all imperfects, that grew out of the wood. The mycelium that penetrated the agar quickly became oidial and the subsequent developmental process to form fruit bodies was similar to that observed in ME agar cultures. Primordia and fruit bodies were usually formed on the wood block, before they formed on the agar. Oidia were rarely found on the mycelium on the block surface. The hymenium that emerged to cover the wood surface was compact and tough owing to the abundant skeletal hyphae. Careful dissection revealed that the differentiation of generative hyphae occurred inside the tracheids to a depth of about 3-6 mm. The composition of the mat was similar to primordia formed in nature but had many more binding hyphae. The primordia were localized on certain positions of the block in contrast to the complete covering of hyphae on blocks that had been inoculated and placed under similar conditions. Pigmentation of the hyphae was observed only in the light and in areas where primordia were formed. A diffusible pigment was noted as the cultures aged, particularly beneath the wood and fruit bodies on the agar. The hymenophores of the fruit bodies formed on pre-infected wood were more normal in appearance in that a daedaloid hymenial conformation was produced, Figure 59, page 137. Many of the fruit bodies were raised and became appressed against the surface of the lid. The mycelium grew longitudinally to form a pileus-like lip. The hymenophores continued



to be irregularly branched and fused except at the extreme margins where they remained as evenly spaced projections, Figure 60, page 137.

The mycelium emerging from wood blocks in the dark was snow white and lightly fasciculate on the surface of the mats that were formed. Hyphal differentiation was evident in that non-pigmented, aseptate, elongate cells, similar to skeletal hyphae in early stages of development, had formed. Generative hyphae were abundant and were found as leading hyphae on the surface of the mats. Oidia were common in the agar but again were sparse or lacking on the wood.

The cultural environments provided in all experiments to this point in the study failed to induce the formation of natural basidiocarps. Decidedly atypical fruit bodies were formed when the following conditions were provided: a rich medium, either natural or artificial, to support vigorous vegetative growth; a plentiful supply of moisture in the medium; a continuous, high relative humidity surrounding the aerial mycelium; still air or aeration, either by direct exposure to the ambient conditions of the laboratory, or a forced air flow; light of high or low intensity and of variable duration; a temperature range near the optimum range for vegetative growth. The low pH of the medium and the removal of the vegetative mycelium from direct contact with a moist medium had no apparent effect on fruiting responses. Fruiting was vigorous in most cases and viable basidiospores were produced. Surprisingly, stock cultures on ME agar refrigerated at 10° C and exposed to only brief periods of illumination, were found to form fruit bodies.

There were however, two environmental factors which invoked a formative response by the mycelium, suggesting that they were important in the development of normal basidiocarps.





The first factor was the nature of the wood itself. Naturally infected wood blocks or wood blocks inoculated by the Tamblyn-DaCosta method were internally decayed by the vegetative mycelium before the appearance of any surface growth. All mycelium which passed from the tracheids into the ambient conditions on the surface was composed of generative and skeletal hyphae. Primordia were compactly interwoven and firmly attached to the substratum by numerous skeletal hyphae. Strand-like associations of skeletal and generative hyphae were evident where the mycelium emerged from the end of the tracheids. Few oidia were formed even in humid conditions, a situation interpreted as a physiological condition in the generative hyphae imposed by the environment of the wood substratum. Oidia formed when generative hyphae grew from the wood onto nutrient agar.

In contrast, wood blocks which were inoculated with mycelium placed on the surface became covered with a dense mat of loosely constructed hyphae. The mycelium lacked extensive hyphal differentiation to form skeletal and binding hyphae and the generative hyphae frequently fragmented to produce oidia. In very moist conditions the primordia were easily stripped from the wood since both reproductive and vegetative mycelium were almost entirely superficial.

The second factor involved the moisture conditions provided by the Tamblyn-DaCosta apparatus. A nearly normal basidiocarp was formed when water was periodically added to the wood block to provide a continuously changing relationship between atmospheric humidity and moisture in the substratum. The evaporation of water from the cotton provided a high relative humidity near the lower portion of the block where several primordia and the basidiocarp developed. The slight internal zonations





of the primordia were indicative of the fluctuating atmospheric conditions of the laboratory and the periodic provision of moisture to the substratum. Strand-like associations were abundant in the primordia and no excess moisture was present in the basidiocarp mycelium.

Directed by the analysis of the results reported above I designed the final two experiments to demonstrate that typical basidiocarps could be induced to form from pre-infected wood by altering the moisture conditions of the cultural environment. A favorable temperature for vegetative growth and diffuse light for initiation of reproductive growth were supplied in the manner outlined in the methods section.

## (2) Collection 100

A tray was filled with water to cover 1/4 inch of the lower surface of the wood block. Mycelium emerged from the ends of the cross-sectioned tracheids immediately above the surface of the water. An elongate primordium with an enlarged, club-shaped terminal end formed in five days. Irregularly branched and fused hymenophores emerged to cover the entire surface. Short tooth-like projections developed on the upper surface in the place of a pileus. The hymenophores on the lower surface were daedaloid while those on the lateral surfaces were variously branched with either sharp, clubbed or flattened and fan-like apices. To provide less humid conditions, the block was raised out of the water and placed on moist filter paper. The tray was covered with a translucent polystyrene sheet to prevent rapid evaporation. An opening was left at one end of the tray to expose the basidiocarps to fluctuations in the atmospheric conditions of the laboratory. The severity of the dry air (the relative humidity varied from 35-59%) was modified by the evaporation of water from the filter paper. The filter paper was allowed to





become dry on the surface and then was saturated with water. After three weeks of alternate wetting and drying of the filter paper, a pronounced change was noted in the basidiocarp morphology. The upper surface had become more or less smooth with the addition of mycelium to cover most of the tooth-like projections. The terminal portion of the basidiocarp expanded and became somewhat flattened to provide a covering over the hymenial elements on the undersurface. The hymenium was lamellate at the center and irregularly toothed and branched at the margins.

The alternate wetting and drying of the substratum appeared to allow a more normal fruiting response. The culture was then returned to the constant humid conditions provided at the beginning of the experiment. In ten days, growth at the margin was observed as numerous irregularly branched hymenophores with a negatively geotropic orientation. The modifications in the morphology of the basidiocarps induced by changing moisture conditions can be clearly seen in the illustrations of Figures 61 and 62, page 139. A series of sections through the basidiocarp revealed definite strand orientation in a horizontal direction above the normal lamellae. The directional orientation was not present at the margin where the irregular hymenophores developed.

### (3) Collection 122

A more satisfactory manipulation of moisture conditions was achieved in the fume hood. Moisture was directly applied to the logs without raising the ambient relative humidity of the chamber above 70% for any extended period of time. Abnormal fruit bodies formed after the initial application of moisture but additional growth did not occur even when the interior of the logs was moist.





The relative humidity of the chamber was raised to 80-100% using the aspirator bottles and closing the door to the chamber. The humidity level was maintained for two consecutive days and then allowed to return to the ambient room conditions by opening the door. The logs were allowed to dry on the surface and then were moistened and the relative humidity again raised. Thereafter, moisture was periodically applied every morning and evening. After 14 days, normal basidiocarps had formed on several logs. They had a typical hymenial conformation as seen in Figure 63, page 139. A macrozone formed on the surface of several basidiocarps after the provision of a prolonged dry period for six days, followed by the resumption of alternate wet and dry conditions. The appearance of a pileus with a zone is illustrated in Figure 64, page 139.

The growth zone is not as distinct as those found in nature. It was evident that the low light intensity and moderate temperature provided in culture failed to induce the heavy pigmentation and abrupt termination of growth so evident in the more severe conditions in the natural environment. Serial sections of the pileus revealed a distinct macrozone in the context, contiguous with the growth zone on the surface. Microzonations were indistinct and it was not possible to correlate the number of zones with the controlled moisture fluctuations. Strand-like associations of generative and skeletal hyphae were horizontally oriented in the direction of growth. The development of the basidiocarp was therefore similar in most respects to those found in the natural habitat.

Several small blocks of wood from collection 119 were placed in the fume hood. One of these formed a normal basidiocarp. It was covered with a bell jar and continuously moistened to provide a high relative





humidity. The growing margin of the basidiocarp became abnormal. The elements of the context and hymenophores failed to respond in a geotropic fashion but turned vertically upward. I was unable to tell if the curvature of the margin to humid conditions was positively phototropic or negatively geotropic. As seen in Figure 65, page 139, the new growth was on the upper surface of the pileus rather than the lower surface. The lamellae remained unchanged on previously formed portions of the basidiocarp, Figure 66, page 139.

Continuous favorable conditions for development of basidiocarps did not result in a constant increase in basidiocarp size. For some reason, apparently internal, growth stopped and in several cases did not resume. This phenomenon has also been reported in experiments with *Lenzites betulina* by Lohwag (1955) and with *Fomes levigatus* by Corner (1932b).

Figure 48. Hyphae grown on non-nutrient agar bearing fertile basidia. (X1000)

Figure 49. Cystidium and inflated cells on hyphae grown on non-nutrient agar. Extracellular concretions are present at the base of the cystidium. (X1000)

Figure 50. An aggregation of hyphae to form coils from which branch hyphae radiate in all directions. (X2000)

Figure 51. Cross section of a dikaryotic fruit body showing a strand-like association of generative and skeletal hyphae. (X360)

Figure 52. Aggregation of marginal hyphae of a primordium to form vertically erect fascicles. (X300)



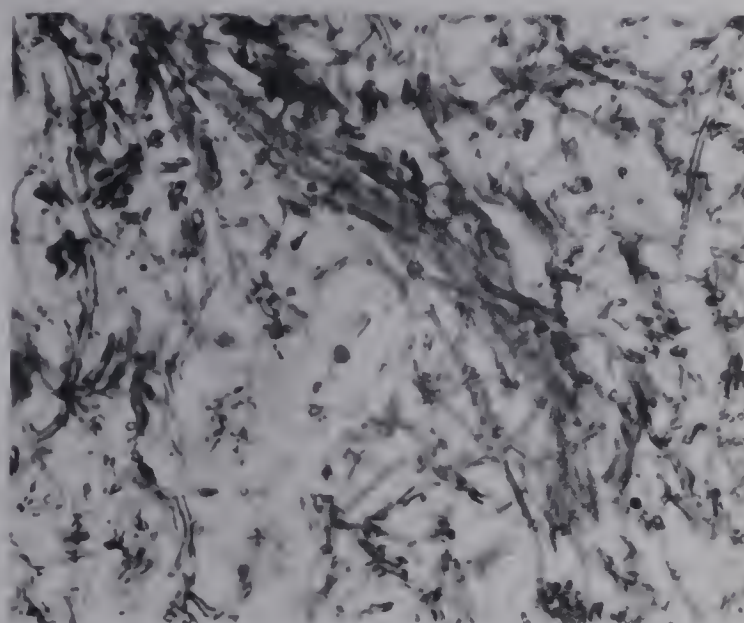
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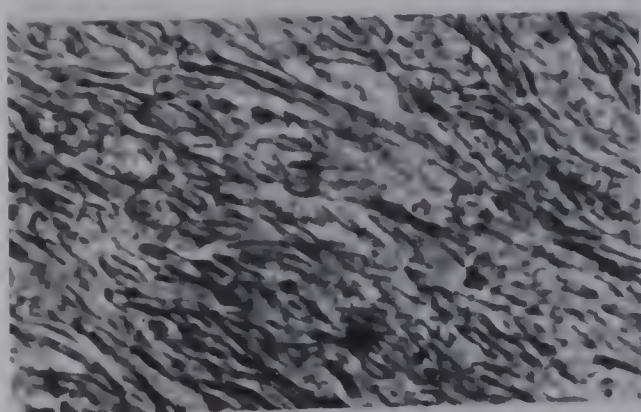
- Figure 53. A "lettuce-like" fruit body on malt extract agar. The irregularly branched and serrated hymenophores can be seen to arise from a small basal primordium. (X1)
- Figure 54. Small, individual or fused hymenophores that are stalked and variously branched, arising from a large primordium. (X1)
- Figure 55. Longitudinal section of a hymenophore formed in culture showing the compact nature of the hyphae. (X1000)
- Figure 56. Transverse section of the hymenophore illustrated in Figure 55 showing the cross sectioned ends of the skeletal hyphae. (X1000)
- Figure 57. Monokaryotic fruit body showing the irregular arrangement of basidia on marginal hyphae. (X200)
- Figure 58. Multinucleate condition of oidia formed by fragmentation of a monokaryotic vegetative mycelium. (X1700)
- Figure 59. Fruit body which has developed from a pre-infected wood block. Note the daedaloid appearance of the hymenophores. (X4/5)
- Figure 60. Upper surface of the fruit body illustrated in Figure 59 showing the orientation of the hyphae due to the presence of the lid of the petri plate. (X4/5)



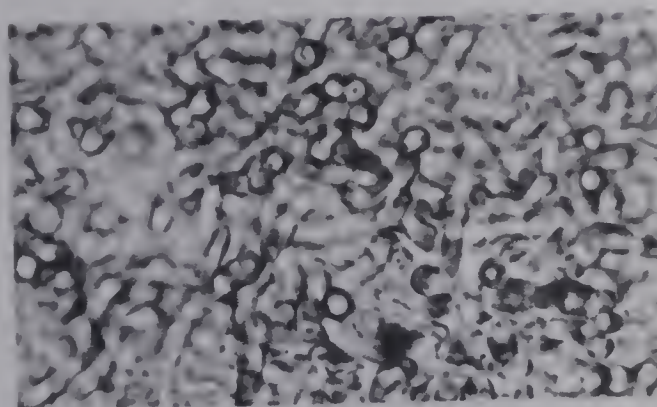
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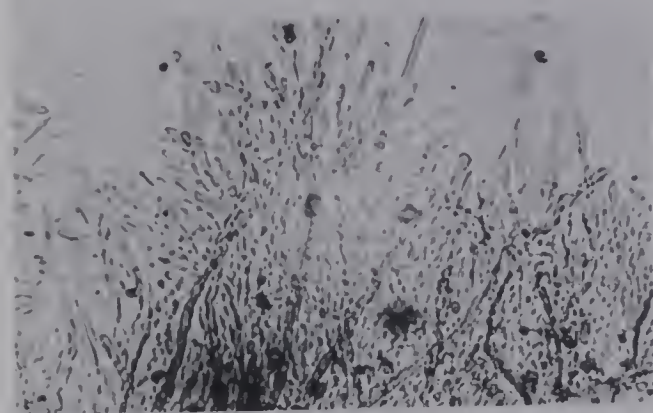
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- Figure 61. Lower surface of a fruit body formed on pre-infected wood. The central part is lamellate while the marginal portion shows a daedaloid conformation. (X1)
- Figure 62. Upper surface of the fruit body illustrated in Figure 61. Note the irregular projections near the base, a dark, uneven surface developed over previously formed hymenophores and the upturned margin of irregularly branched hymenophores. (X1)
- Figure 63. Lower surface of a pileus showing normal lamellate hymenophores formed under cultural conditions. (X1.4)
- Figure 64. Upper surface of the basidiocarp illustrated in Figure 63. Note the macrozone on the margin. (X1.4)
- Figure 65. Lower surface of a pileus formed on pre-infected wood in culture. (X3/5)
- Figure 66. Upper surface of the pileus illustrated in Figure 65. Note the irregular nature of the hymenophores that have turned vertically upward in response to conditions of high relative humidity. (X3/5)





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## DISCUSSION

The Environment and Developmental Morphology

"Our classification of these fungi can never be complete from even a simple morphological basis until the development and hyphal systems of the basidiocarp are known for each species." (Smith, 1966, page 153) The results of this study provide, I believe, a better understanding of the development of *G. saepiarium* in the natural and cultural environments. Information to elucidate the influence of environmental factors on this development has also been obtained. The first part of this discussion deals with the development of the mycelium and the basidiocarp in the natural environment.

The aspects of development are relatively uniform where environmental conditions, characteristic of the habitat of *G. saepiarium*, are not extreme. Macroscopic and microscopic characters of basidiocarps, collected throughout the range of distribution of the species in North America, were remarkably similar. Structural differences were restricted, for the most part, to portions of the mycelium most exposed to the external environment. Thus, under 'normal' conditions the following sequence of events in the development of a basidiocarp from the vegetative mycelium is found to occur. The vegetative mycelium, active in wood-decay and characterized by medallion clamps in this species, becomes differentiated as it emerges from the protective confines of the wood tracheids. Secondary branching greatly increases and generative hyphae which possess normal clamp connections form terminal thick-walled aseptate cells, the skeletal hyphae. Groups of generative and skeletal hyphae in strand-like associations accumulate on the wood



surface, arising in all directions to form a hemispherical primordium. The primordium exhibits differential growth rates on the upper and lower lateral surfaces attributable to hyphal differentiation. Apical hyphae on the upper surface, which are exposed to insolation and low relative humidity, form skeletal hyphae which become aggregated into fascicles. Hyphae on the lower surface differentiate to form reproductive cells, most probably, in response to higher relative humidity and lower light intensity provided by the shelter of surface hyphae above. Strand-like associations in the center of the primordium continue to grow in a horizontal fashion between the upper and lower regions of differentiation to form a marginal rim or pileus. Concomitant with the formation of the pileus, fertile hymenophores issue from the lateral surface of the primordium and from the lower surface of the pileus to form the typical lamellate hymenial conformation of the species. Continued growth of the pileus and lamellae is intermittent, slowing when the relative humidity and substrate moisture is low and continuing when high moisture relationships are reestablished. Severe, periodic drought results in macrozonations on the pileus surface, while the moderate diurnal fluctuations usually result in microzonations in the context which are not apparent on the pileus surface. The zones represent the differentiation of apical cells of generative hyphae to form skeletal hyphae in response to moderate desiccation and insolation.

The regular disposition of hymenophores is related to the regular horizontal arrangement of the strand-like associations of generative and skeletal hyphae in the context of the pileus. The composition of the hymenophores differs from that of the context in having hyphae of a smaller diameter that are more compactly interwoven and in possessing





less inconspicuous strand-like associations. Lamellate hymenophores descend vertically downward in a geotropic fashion. As the pileus expands in a radial fashion, additional lamellae are formed at the margin to maintain equal numbers per surface area. The variability in the branching and fusion of individual hymenophoral plates and the uniformity of their vertical length is apparently controlled by internal factors. External factors can modify hymenial conformation to some extent, particularly when the orientation of strand-like associations which respond to environmental factors is altered.

The hymenium is formed on the outer surfaces of the lamellae and lower surface of the context by the abrupt termination of lateral growth of skeletal hyphae, whose progress is directed either vertically downward in the lamellae or horizontally outward in the context. The uniform intervals between the individual lamellae appears to be internally controlled. The hymenial elements, the basidia and cystidia, are found immediately behind the growing margins of the lamellae and the pileus. In this position they are irregularly situated, the hymenial layer not yet established. The apices of elongate generative hyphae often enlarge to form a basidium with basidiospores. It appears that the stimulus for the transformation of one kind of cell to a specialized element occurs immediately after differentiation of leading terminal hyphae. In the older portions of the hymenium a regular palisade of hymenial elements is formed. Basidia remain as a persistent layer upon desiccation and a new layer is formed over the old layer when growth resumes after dry conditions.

Results of the study of basidiocarp development in the natural habitat indicate that the differentiation of hyphae to form pileate



basidiocarps occurs in direct response to environmental conditions present during development. The fact that the initial control of development in this fungus is of a genetic nature should not be overlooked and will be discussed later. The environment plays a formative role in the execution of the genetic potential.

The formation and orientation of the strand-like associations is an integral part of the normal development of the basidiocarp. In this study, they showed a marked sensitivity to fluctuations of light and the atmospheric humidity. A discussion of the nature of the association of skeletal and generative hyphae provides some important morphogenetic concepts for consideration. Of primary importance is the manner in which skeletal hyphae are formed. The apical cells of the leading hyphae that emerge from the wood stop growing and branches arise from clamps behind the main apex. The significance of this activity is a change in apical dominance whereby lateral branches assume the role of continued hyphal growth. The formerly terminal cell elongates but is determinant in growth and becomes a thick-walled skeletal hypha. Lateral branches, in turn, give rise to the skeletal elements of the next wave of growth. Septation is considered to be important in the transference of apical dominance to lateral branches. The most plausible means by which this action is accomplished is the termination of cytoplasmic continuity by closure of the septal pore. Cytological evidence is needed to verify this theory.

Robertson (1968) demonstrated that any treatment which led to the prolonged arrest of growth in the terminal hyphae of *Neurospora crassa*, also led to the production of additional septa. Apical excision of terminal hyphae resulted in the production of dense tufts of hyphae from





abundant secondary branching. The branches nearest the excised apex did not greatly exceed those arising from nearby excised branches from the same or different hyphal axes. Subsequently, marginal growth of the colony was more or less uniform.

A meaningful application of these results can be made in the case of *G. saepiarium*. The terminal cells which emerge from the wood are exposed to the inhibitory influence of oxidation, irradiation and evaporation. In the absence of physical contact with the substratum, leading hyphae cannot obtain a food supply independently and the nutritional advantage of the apical position is lost. The exposure to external environmental factors represses the normal progress of cell elongation. Clamp connections are formed behind the exposed cell and secondary branching from the clamps allows the continuation of the growth of generative hyphae. A succession of advancing apical cells from secondary branching and the periodic termination of their growth due to fluctuations in the ambient conditions of the environment presents a plausible explanation for the presence of growth zones in the development of *G. saepiarium*. The close association of generative and skeletal hyphae is believed to be due to both the mutual connections provided by secondary branching and the addition of unrelated hyphae to the main axis by the thigmotropic response of hyphae under the influence of a changing atmospheric humidity.

The strand-like associations, which grow at first in an upright position in the primordium, are turned to a horizontal position due to their apparent inability to continue vertically upright in an unchanged state. A microscopic examination of their approach to the surface revealed that the uppermost hyphae were desiccated and formed fasciculate aggregations in response to adverse conditions in an exposed position.



The hymenophores which descend vertically downward have been shown to be positively geotropic. When a basidiocarp was inverted so that the hymenophores were uppermost, the strand-like associations descended through the already differentiated pileus to form hymenophores on the lower surface. A pileus placed in humid conditions with its pileus oriented in a normal fashion failed to initiate growth on the surface but new growth at the margin turned vertically upward. No conclusive evidence was obtained to determine if the response of marginal hyphae was positively phototropic or negatively geotropic.

The structural composition and geotropic response of hymenophores indicate that they are special developmental products. Differences in structural arrangement are most likely influenced by the positional relationships of the strand-like associations in the context and the microenvironment afforded by the pileus. Relative humidity is the factor considered to be of primary importance in influencing the differentiation and orientation of the hyphae in the basidiocarps. The position of binding hyphae in the thick tissue at the base of the basidiocarp indicates that they develop in response to the protection afforded by the surrounding hyphae and to a position adjacent to an abundant nutrient supply. The tardy formation of binding hyphae is reflected in their light pigmentation since their position excludes the influence of solar radiation. The irregular disposition of the strands in apileate and partially pileate basidiocarps in shaded positions in the environment indicated that their normal response to fluctuations of moisture and light was interrupted. Confirmation of this effect was obtained in the analysis of fruit bodies formed under the moist conditions inherent in artificial culture.





When the microstructure of the abnormal, apileate fructifications on liquid and agar media was analyzed, it was found that strand-like associations of hyphae were absent or sparse. When they were present they had no specific orientation. Hymenophores were similar in construction but more irregularly branched and fused than those found in nature. A gradual change from the very atypical fruit bodies on agar and liquid media to normal, pileate basidiocarps on wood blocks was correlated with a similar change to formation of strand-like associations which were regularly arranged.

The following cultural conditions are important in obtaining a normal basidiocarp in culture. Light is necessary for initiation of basidiocarp development, but subsequent normal development will occur under very low or high light intensities of continuous or brief duration. Light enhances pigmentation in the walls of the skeletal hyphae, especially in a dry atmosphere and accentuates the growth zones. A medium providing the nutrients necessary for normal vegetative growth, and a moderate temperature were found sufficient to support a fruiting response. Only in wood media in which the tracheids were left intact were the normal medallion clamp connections found. The abundance of moisture and nutrient material in liquid and agar media caused the vegetative mycelium to fragment and form oidia, asexual reproductive structures which were considered to be indicative of abnormal cultural conditions. Oidia were found on wood media supplemented with nutrients but were absent both on wood without nutrient supplements and wood in the natural habitat. Where oidia were obtained in culture normal basidiocarps did not develop. Temperature, pH and  $\text{CO}_2$  were limiting factors only at extreme levels. The poor utilization by the fungus of cellulose sources supplemented



with vitamins was puzzling and further investigation is needed.

The moisture conditions of the substratum and atmosphere are of primary importance for the support of reproductive growth. It was obvious in the natural environment that the periodic growth increments of the basidiocarp were formed in direct response to changing moisture and temperature regimes. In nature, light, temperature and relative humidity are synchronized in a diurnal fluctuation. The degree to which each is related is modified by climatic conditions during the diurnal cycle. In simulating the fluctuating environmental conditions of the natural habitat in the laboratory liquid and agar media cannot be used but a natural wood substratum can be used.

The significance of alternating the relative humidity of the atmosphere as opposed to that of the substrate is emphasized. All wood block cultures which were provided with continuously moderate or saturated atmospheric humidities failed to form natural basidiocarps. In experiments where the relative humidity was alternately changed from 100 to 30% and the wood kept continuously moist the normal formation of basidiocarps occurred. Saturation of the wood prevented vegetative growth because of low oxygen concentrations. This result is in contrast to those of Hopp(1938) and Plunkett(1956). They were able to obtain normal fructifications using constant relative humidities in aerated chambers. The provision of an alternating relative humidity, such as that described in this thesis, applied as a modification to the method of Tamblyn-DaCosta should successfully induce the formation of normal basidiocarps in poly-pores which exhibit a growth pattern similar to that of *G. saepiarium*.

Several aspects of the mycelial characteristics found in culture merit additional discussion. On agar media the development of reproductive





elements, the basidia and basidiospores, was not restricted to mycelium bearing clamp connections. The formation of supposedly monokaryotic fruit bodies and poorly developed fruit bodies from incomplete mating reactions is considered to be a phenomenon that is permitted, if not fostered, by cultural conditions. No basidiocarp, pileate or apileate, collected from the natural environment was found to lack clamp connections on the generative hyphae. The absence of typical skeletal and binding hyphae in monokaryotic fruit bodies suggests that these fruit bodies would not be capable of surviving under natural environmental conditions. It would be interesting to know if the multinucleate condition found in the vegetative cells of monokaryotic and dikaryotic mycelium which produce fertile fruit bodies exists in a natural situation. The artificial environmental conditions of culture, as implied by the presence of oidia, may be important in influencing unusual nuclear behavior. The nuclei were consistently concentrated in the hyphal tips and most abundant in the oidia. However, King and Alexander (1969) reported a consistent multinucleate condition in terminal cells of *Alternaria solani* and suggested that they were possibly heterokaryotic. This possibility exists in *G. saepiarium* in view of the extreme morphological variability encountered when studying colonies arising from germinated oidia.

Of outstanding significance was the association of fertile, fruit bodies with clamp connection formation resulting from compatible pairings of monosporous mycelia in culture. Incomplete dikaryotization of the mycelium and therefore few clamp connections resulted in poorly developed fruit bodies and primordia. Negative pairing reactions occasionally resulted in raised mats of mycelium but lacked the differentiation of





hyphae present in the other pairings. Raper (1953) and Aschan (1954) have reported similar fruiting responses in *Schizophyllum commune* and *Collybia velutipes* respectively. It should be kept in mind that both of these species are reported to have a tetrapolar type of interfertility while *G. saepiarium* is reported to have the bipolar pattern (Mounce and Macrae, 1936). Also of importance is the fact that certain isolates fruited more consistently in all pairings, indicating as did the ability of monokaryons to fruit, that fruit body formation is not necessarily related to sexual incompatibility factors.

The exact genetic basis of clamp connection formation in the pairings which I made remains uncertain. The study was of a preliminary nature and further work using larger numbers of monosporous progeny in mating experiments is needed. Problems in carrying out the necessary genetic crosses to establish mating type include the rapid staling reaction produced by some isolates on malt extract, the slow process of dikaryotization, and the possibility of contamination of paired cultures by oidia.

The formation of clamp connections was often irregular in cultures regardless of inoculum source. The absence of a clamp-forming response induced by staling, anaerobic and poor nutrient conditions was reversible if the mycelium was provided with continuously renewed nutrients and air. Butler (1968) observed a similar environmentally induced change in clamp formation which was reversible in *Coprinus disseminatus*. It is of some interest to find that the formation of basidia and cystidia on hyphae growing on non-nutrient agar took place in the absence of clamp connections. In no case was the vegetative mycelium within wood found to bear hymenial elements. False clamps were found on the generative hyphae in





the wood as well as in the mycelial strands and plates. The discovery of both clamps and pseudoclamps on fossil mycelium within wood of a fern from the Middle Pennsylvanian may be noted here (Dennis, 1969). The frequent occurrence of pseudoclamps in some fungi may not be strictly associated with a sexual phenomenon but may be attributed instead to an environmentally induced response of the vegetative mycelium.

### Taxonomic Considerations

The analysis of macroscopic and microscopic characters of mycelium and basidiocarps in culture and in nature allowed an evaluation to be made of characters commonly employed in the taxonomy of *G. saepiarium* and related species.

#### A. Characters in the natural basidiocarp

The work with a large sample of basidiocarps, collected in a variety of habitats, extended considerably the range of many characters described in other papers (Murrill, 1908; Rea, 1922; Overholtz, 1953). Hyphal systems were not sufficiently different in species of *Gloeophyllum* to allow species separation. The use of this character is of value in separating closely related genera. No other new characters were found in the study of basidiocarps which could be added to those already published. The common practice of the separation of species on the basis of one or two characters has not led to a satisfactory diagnosis for the species of *Gloeophyllum*. For example, the prime characters used by Overholtz (1953) are dimensions of the spores and context which according to the present data render his key unworkable. It has been found that a complex of characters must be considered before an adequate segregation





of species of this genus can be made (see Table II, page 36). Characters considered to be of importance include hymenial configuration, color of the context, presence and abundance of cystidia, thickness of the margin, nature of the tomentum, and the physiognomy of the pileus.

Species of *Gloeophyllum* occupy similar habitats and therefore have similar characteristics as a result of their adaptive responses to the environment. Their characters overlap but they have, within a range, a distinctive appearance. It is difficult to distinguish between species by individual microscopic features but when the total of the microscopic features which contribute to the macroscopic appearance is considered, the differences become apparent. For the most part, individual characteristics during development under a variety of environmental circumstances were consistent in their expression. Some features which may lead to confusion are discussed below. The hymenium in individual species often varies but the thickness of the hymenophores and the extent to which they fuse to become daedaloid or poroid is relatively consistent and is a valuable character. Hymenial conformation as a taxonomic criterion at the family or generic level should not be used because similar manifestations of hymenial conformation are represented in other genera. Variability in the elements of the hymenium was observed in basidiocarps of *G. saepiarium* that were not immediately preserved upon removal from the substratum. Basidia have been found to germinate in specimens stored in cool moist containers. Elongate tips on cystidia-like elements formed when the basidiocarps were slowly dried. A large sample of basidiocarps should be examined when determining the microstructural characteristics of the hymenium and care must be taken to prevent changes due to exposure to unusual environmental conditions.





The immature margin of *G. saepiarium* is often brightly colored and is considered to be a distinctive feature of this species. However, basidiocarps that are not exposed to direct sunlight often fail to produce this character. Since each species has, in general, a distinctive coloration, pigment analysis may provide a useful taxonomic criterion to replace the subjective evaluation now used.

The species of *Gloeophyllum* are genetically isolated and morphologically similar. Where the morphological characteristics overlap, their intersterility has been found to be a reliable basis for separation.

#### B. Cultural characters

Some of the problems in culture are similar to those that plague a classification system based on features of the basidiocarp. Cultural characters are useful in separating genera but, in the case of *Gloeophyllum*, species differences are difficult to determine. The characters manifest in culture are variable according to the inoculum source and cultural conditions. Even under the standard conditions employed, the interpretation of the different characters used by Nobles (1965) to delimit taxa, presented a problem.

In artificial culture morphological features of the basidiocarp failed to reflect the precise hyphal structures found in nature, although there were counterparts. There is also a great difference in the characters expressed by different isolates in culture. The differences between characters in artificial culture and those in nature diminished as cultural conditions were modified to simulate normal environmental conditions. This situation points out the necessity of providing standard cultural conditions. Of taxonomic importance are the characters which



remained unchanged in all environments and the characters that were consistent under defined cultural conditions. These characters are discussed below.

The major criteria selected for discussion are those used by Nobles (1965) to distinguish *G. saepiarium* from other polypores. Negative results in tests for extracellular oxidase, a positive reaction upon the application of KOH to pigmented mycelium and basidiospore characters were the only features found to be consistent in all environmental situations. Pigmentation was associated with differentiated hyphae if growth took place in the light. The recognition of cultures which lack differentiated hyphae but which exhibit color is an apparent contradiction in Nobles key. The confusion here may be due to the definition of differentiated hyphae. I would define differentiated hyphae as elongate, terminal cells which lack septa and cytoplasm and possess colored walls. Hyphae which are colored but have pigmented material in the lumen are considered to be products of a staling reaction and not of morphological differentiation. Coloration of the reverse plate in malt extract media was a common reaction not reported by Nobles. The characteristics of skeletal and binding hyphae in culture were quite variable and much less uniform than those found in nature. Extreme caution is recommended when attempting to use them for classification purposes. Similarly, the presence of clamp connections on the generative hyphae is not consistent in *G. saepiarium*. It can be said that, in general, *G. saepiarium* bears uniform clamp connections on the mycelium which produces abundant fruit bodies.

I have found, as has Nobles, that *G. saepiarium* has a distinctive odor in culture which may be described as apple-like. The characteristics





of basidia and cystidia were subject to structural modification in developmental stages by environmental conditions. Again caution is advised in placing undue emphasis on their nature when only a small sample is available. Oidia were produced in culture but were not observed in the natural environment. They represent the tendency of the vegetative mycelium to fragment in the artificial conditions of agar culture. In as much as the mycelium of other polypores remain intact under similar cultural conditions, this feature is useful in classification.

The fruit bodies which develop in culture are usually morphologically distinct for the genus *Gloeophyllum* but member species cannot be separated on characters of basidiocarps. The separation of *G. trabeum* from *G. saepiarium* on the basis of cultural characteristics is often difficult, if not impossible. The cultural characters and the characters of the natural basidiocarp of *G. odoratum* are similar to those of *G. trabeum* and *G. saepiarium*. This similarity justifies its inclusion in the genus *Gloeophyllum* as has been done by Imazeki (1943) and David (1968).

#### Comparative Developmental Morphology

In this section basidiocarp development of *G. saepiarium* is compared with that of other polypores whose development has been described. The environmental stimuli required to induce a normal fruiting response in *G. saepiarium* are different from those found to be important in the development of three stipitate polypores, *Polyporus squamosus*, *Polystictus xanthopus* and *Polyporus brumalis*.

Buller (1909) found, in studying *P. squamosus*, that light was necessary to stimulate the formation of a primordium but that further



development to form a mature stipitate basidiocarp could take place in total darkness. The stipe was found to be negatively phototropic since it did not respond in any stage of its development to unilateral illumination. The pileus formed was negatively geotropic and the hymenophores were positively geotropic.

Corner (1932a) studying *P. xanthopus* reported the stipe to be positively phototropic since it responded to unequal illumination. No tissue was found in the stipe that could account for the change in direction brought about by inflation of cells. Therefore, he postulated that the hyphae at the growing margin of the stipe were the responsive elements. The stimulus to form a pileus was not determined, but it was noted that as a lateral outgrowth of hyphae occurred at the apex, a crust formed on the uppermost surface of the pileus so was believed to prevent further upward growth by acting as a mechanical stay. The marginal hyphae were therefore directed to a position at right angles to the force of gravity. Basidiocarps formed under humid conditions provided by a bell jar and periodic wetting of the wood substratum. The basidiocarp revived after several days of desiccation, but prolonged drought was fatal.

Plunkett (1961) has shown that light and low humidity are necessary for normal pileus development in *P. brumalis*. The stipe was found to be positively phototropic initially but later became negatively geotropic after formation of a pileus. A light intensity of 160 foot-candles was required to induce normal pileus development. When both stimuli were present the phototropic stimulus had an overriding influence on the geotropic stimulus. Plunkett also reported that stipe elongation of *P. brumalis* was brought about by both direct growth of the apical





hyphae and inflation of subapical hyphae. The greater portion of stipe length was found to be due to subapical growth that occurred after pileus initiation. The pileus was always oriented toward the strongest light source, a response similar to that of *P. xanthopus*. The continuous aeration of cultures to provide increased evaporational rates strongly favored pileus development. This observation prompted Plunkett to postulate that pileus formation was, at least in part, an aerotropic response.

Fruiting in *G. saepiarium* was photoinduced but high light intensities were not required for basidiocarp development. Differential growth rates on the upper surface and lower sides of the primordium resulted in a horizontal orientation of the marginal mycelium to form a pileus. In contrast, a uniform vertical elongation of a hemispherical primordium to form a tubular stipe occurs in the species described above. *G. saepiarium* failed to form pileate basidiocarps under constant relative humidities, high or low, regardless of aeration. Low relative humidity repressed both vegetative and reproductive growth. The initial inhibition of growth on the upper surface of the primordium and subsequent horizontal orientation of the mycelium occurred in response to alternating relative humidities. Only later, after marginal growth was directed horizontally did a geotropic response of the hymenophores and negatively geotropic response of the pileus become evident. Growth was direct in all stages of development.

The growth mechanics of *Fomes levigatus*, a bracket fungus described by Corner (1932b) were similar to that of *G. saepiarium*. However, the structure of the basidiocarp was different. A thick crust was formed on the upper surface of the pileus, the context formed was much deeper



and no binding hyphae were found. Microzones present in the context, correspond with the daily increments of marginal growth. Macrozones were indistinct, probably owing to the fact that the basidiocarps were not able to withstand desiccation for more than a few days.

The differences in the development of basidiocarps which have similar external morphologies indicate that fungi do not achieve their form by utilizing the same developmental pattern. In nature, there appear to be a small number of basic plans, by which fungi can make adaptive modifications in a manner most efficient for survival and reproduction. The adaptive responses of the fungus are to a large extent shaped to the influence of the environment which surrounds it. This situation is clearly pointed out by the comparison presented above. The presence of motor tissue, the ability to develop under conditions of low relative humidity, and the requirement of high light intensity indicate the relationship of *P. brumalis* with the agarics. *P. xanthopus* exhibits the xerophytic nature common in most polypores and is capable of withstanding moderate desiccation. *F. levigatus* forms a thick protective crust which prevents excess evaporation. The ability of both of these species to grow in moist conditions but otherwise succumb to extended periods of drought is probably a reflection of the tropical habitat in which they are found. *G. saepiarium* exhibits adaptations to a changing and often severe environment that characterizes its habitat. The developmental pattern is geared to the fluctuating moisture conditions and the structure of its basidiocarp is such that it is able to withstand extended periods of desiccation.

The contrasting nature of the xerophytic adaptations of the pileus in a *Fomes* species and a *Gloeophyllum* species may also be related to





the type of hymenium exhibited. Buller (1909) reasoned that because of the minute diameter of the pores of most *Fomes* species, the tubes must adhere closely to a position vertical to the force of gravity to effectively disperse their spores. The thickness and therefore the rigidity of the context was thought to be functional in maintaining the position of the tubes. In contrast, the pileus of *G. saepiarium* lacks a crust and is composed of loosely interwoven hyphae in a relatively thin context. It is therefore flexible and its position is more liable to change by mechanical or biological forces. The lamellae allow a more efficient spore dispersal in situations where changes in the geotropic orientation of the hymenophores occur.

It is of interest to note that *G. striatum* is the most consistently lamellate species of the genus and has a thin, flexible pileus. Other members have an increasing tendency to exhibit a daedaloid hymenial configuration and a generally thicker context. *G. odoratum* has a predominantly poroid surface and the thickest context of any species.

The differences in growth pattern and response to environmental stimuli of the fungi discussed above indicate the necessity of providing, in culture, those conditions which induce normal basidiocarp development. It is to be expected that polypores which exhibit a growth pattern similar to that of *G. saepiarium* will develop normally under the conditions found necessary to direct the growth of this species.

The careful study of the developmental patterns as well as the microstructural detail is suggested as a useful and necessary approach to the understanding of natural relationships among the polypores and the evaluation of taxonomic characters used in their classification.



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## A P P E N D I C E S



## APPENDIX I

CLASSIFICATIONS OF FRUITBODIES TRANSLATED AND MODIFIED  
FROM R. FALCK (1909, p. 2-25).

1. Classification of fruitbody according to symmetry and form.

I. Radially or bilaterally symmetrical - single or compound due to the fusion of several fruitbodies.

A. Pileate, substipitate or on a ridge-like cushion; round, oval to oblong elliptical (then bilaterally symmetrical) = substipitate forms.

1. Radial form and round.

a. Hymenium on outside of infundibuliform pileus = kratereloid form.

b. Hymenium on inside of a flattened bell-shaped pileus, pendant or lateral = cephelloide form.

2. Bilateral form and elongate.

a. Hymenium exposed = bilateral brackets.

b. Hymenium inside of upper and lower halves of pileus = effused-reflexed forms.

B. Apileate sessile or resupinate.

1. Hymenium exposed, disk-shaped varying in contour from round, oval to oblong-elliptical = resupinate form.

II. Dimidiate but divisible by plane of symmetry.

A. Substipitate, the lateral pileus more or less divisible into radially symmetrical right and left halves = conchate form.

B. Semicircular or oval with broad bases laterally attached with radial lamellae = flabelliform or dimidiate brackets.

C. Elongate linear pilei (often formed due to fusion of several smaller ones), lamellae parallel = laterally elongate brackets.

III. Irregularly developed, not divisible by plane of symmetry.

A. Toothed, spined or sublamellate forms on a regularly formed pileus = imperfect forms.

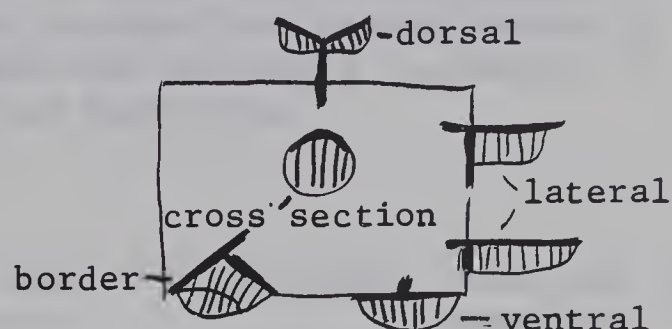




- B. Entire lamellae, spines or teeth attached to a resupinate mycelial mat, occasionally the upper portion forming a normal pileus = "crustenformen".
- C. Apileate irregularly stalked and branched often without a hymenial covering = "monstrosen" (abnormal) forms.

2. Classification of fruitbody according to positional relationships on substratum. (I have included the lateral form with resupinate mycelial plate in this classification. Falck considered it abnormal.)

- A. Surface form - lateral side
- B. Ventral form
- C. Border form - (groove of wood)
- D. Superficial form - dorsal
- E. Cross section form



3. Developmental classification of the fruitbody.

I. Simple or individual construction units.

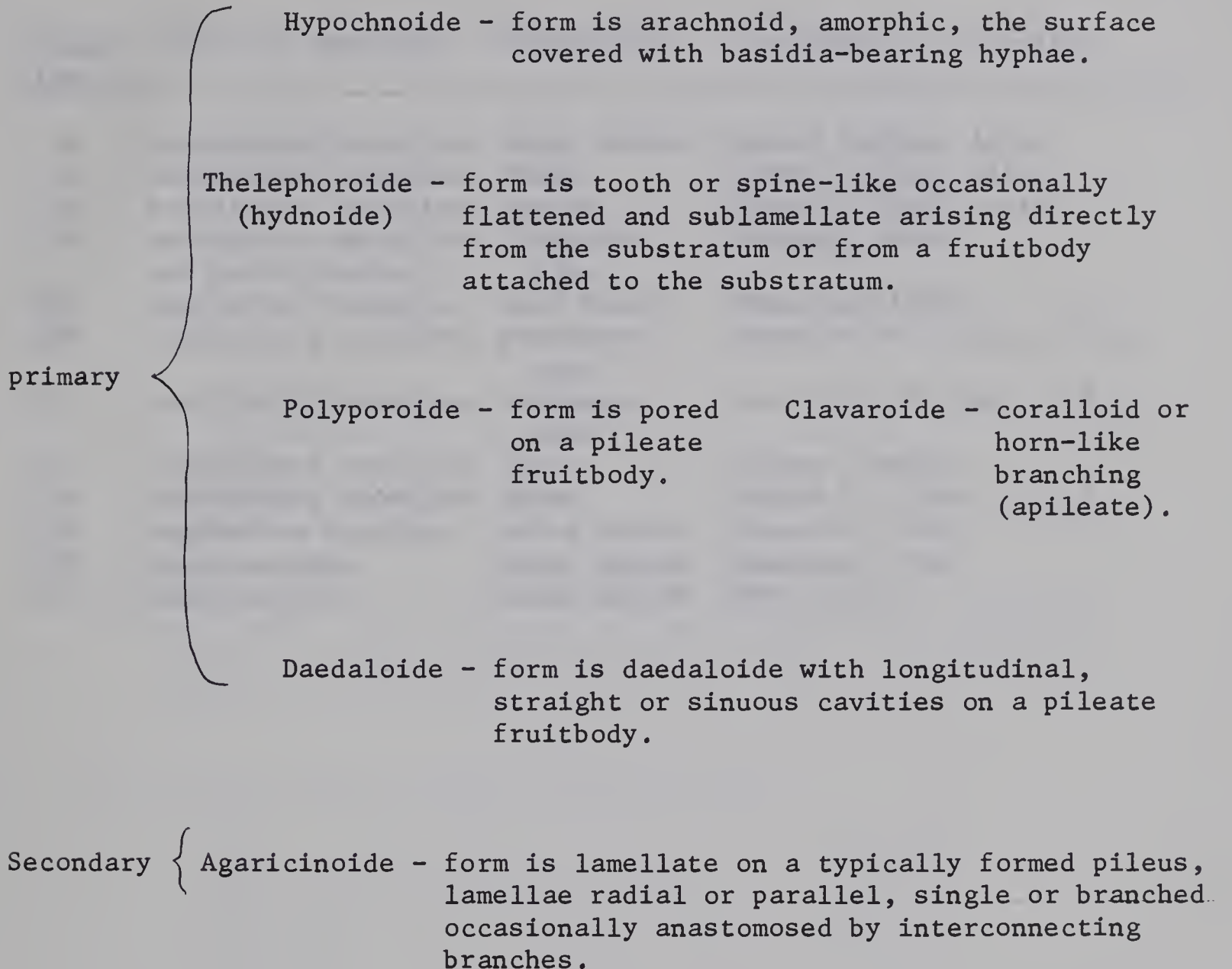
- A. Primary fructification - tooth-shaped hymenophore.
- B. Secondary fructification (a) ventral hymenophore - disk.  
(b) cross section hymenophore - shell.

II. Compound form of construction.

- A. Initial fusion of fruitbody elements before an individual basidiocarp is produced = progenetic form.
  - 1. Constructed from primary units - a toothed mat.
  - 2. Constructed from secondary units - spalten platten formation (bracket, conch, dual fruitbodies one on either side of the primordium).
- B. Fruitbodies grown together only after their establishment as individuals = metagenetic.
  - 1. Those fruitbodies successively formed with primary units on the inside, secondary formation at the periphery (zonate growth of preestablished basidiocarps).
  - 2. Those fruitbodies simultaneously formed beside one another and fusing together.
    - a. Without a common growth zone - true metagenetically fused fruitbodies - (connate).
    - b. With a common growth zone - falsely fused fruitbodies (unequal growth on a preestablished margin).



4. Ontogenetic classification. Morphological appearance of the hymenium classified as developmentally primitive (primary) or advanced (secondary).







## APPENDIX II

A list of the collections of *G. saepiarium* used in the cultural study.

Collec- tion No.	Type of Inoculum	Substratum	Geographical location.
46	basidiocarp mycelium	white spruce	Turner Valley, Alta.
50	basidiocarp mycelium	aspen	Turner Valley, Alta.
60	basidiocarp mycelium	spruce	Allstone Creek, Alta.
63	basidiocarp mycelium and basidiospores	lodgepole pine	Nordegg, Alta.
100	vegetative mycelium	wood block	Edmonton, Alta.
109	basidiocarp mycelium	ponderosa pine	Greenley Co., Ariz., U.S.A
113	basidiocarp mycelium	lodgepole pine	Grant Co., N. Mex., U.S.A.
114	basidiocarp mycelium	pine	Durango, Mexico
116	basidiocarp mycelium	aspen	Carbon Co., Wyo., U.S.A.
119	vegetative mycelium	white spruce	Edmonton, Alta.
122	basidiospores	white spruce	Edmonton, Alta.
123	basidiospores	black spruce	Robb, Alta.



APPENDIX III

A list of formulae for media used in the cultural study.

1. Basal Synthetic Medium (Lilly and Barnett, 1951)

KH <sub>2</sub> PO <sub>4</sub>	. . . . .	1.0 gm
MgSO <sub>4</sub> ·7H <sub>2</sub> O	. . . . .	0.5 gm
Fe <sup>+++</sup>	. . . . .	0.2 mg
Zn <sup>++</sup>	. . . . .	0.2 mg
Mn <sup>++</sup>	. . . . .	0.1 mg
Biotin	. . . . .	5.0 µg
Thiamine	. . . . .	100.0 µg
Distilled water to make	. . . . .	1000.0 ml
Glucose	. . . . .	10.0 gm
Asparagine	. . . . .	20.0 gm

Modification: (1) 2 gm yeast extract substituted for thiamine and biotin. (2) 10 gm of either carboxy-methyl cellulose, Whatman's cellulose powder or filter paper cellulose substituted for glucose.

2. Malt extract medium (Raper and Thom, 1949)

Agar	. . . . .	25.0 gm
Malt extract (Difco)	. . . . .	20.0 gm
Dextrose	. . . . .	20.0 gm
Peptone	. . . . .	1.0 gm
Water (distilled)	. . . . .	1000.0 ml

Modification: Omitted agar.

3. Badcock's Accelerator Medium (Badcock, 1941)

Maize meal	. . . . .	2.5 gm
Bone meal	. . . . .	1.5 gm
Potato starch	. . . . .	0.75 gm
Sucrose	. . . . .	2.0 gm
Malt extract	. . . . .	0.5 gm

Add to 100 gm sawdust.

Modification: Yeast . . . . . 0.2 gm







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